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Direct Quantitative Determination of Nicotinamide in Pharmaceutical Preparations

*By J. M. Goodyear and H. W. Murphy**

Since many of the compounded vitamin preparations contain nicotinamide because it possesses certain pharmacological advantages as compared with nicotinic acid, it is highly desirable to have a direct quantitative chemical method for its accurate determination. Colorimetric methods have been described for the determination of "nicotinic acid activity"; however, they are not specific for nicotinic acid but rather general reactions for the pyridine nucleus (1, 2, 3, 4). Moreover, the determination of nicotinamide in the presence of nicotinic acid by the colorimetric method is complicated by the fact that both compounds give the same color reaction (5, 6). The colors produced are said to be additive, but they differ in their intensities, rates of formation, and stabilities. It is necessary to determine the maximum intensity by making a series of readings. The time required for the development of this maximum and its absolute value is greatly influenced by the cyanogen bromide preparation, the *p*H, the temperature, and the influence of extraneous matter present in the reaction mixture. In addition, slight modifications of the manipulative procedure affect the reaction to such an extent that the entire method may be subject to considerable error.

The microbiological method for the determination of "nicotinic acid activity" (7, 8) is unquestionably the most selective, but it does not enable one to differentiate between nicotinamide and nicotinic acid since both display identical activities (8). It has been suggested that the microbiological method may be combined with a chemical treatment, namely, the "Hofmann reaction," to determine the quantities of nicotinic acid and nicotinamide in mixtures (9). Pollack determined in his investigation of the Hofmann rearrangement of nicotinamide that 3-aminopyridine was obtained as the main product, but that other by-products were formed in appreciable amounts (10). This observation has been confirmed by Camps (11). Since it has been definitely established that 3-aminopyridine does not possess "nicotinic acid activity" (12, 13), this method would appear promising. In evaluating the results, however, one has to consider possible interference due to the toxic effects of 3-aminopyridine and other by-products (14) as well as the probability of brominating nicotinic acid to yield inactive derivatives. We have been unable to detect 3-aminopyridine in the final reaction mixture whenever the degradation of nicotinamide was performed as described by the authors (9).

Careful consideration of the methods referred to above indicated that a more convenient and, if possible, selective method for

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the determination of nicotinamide alone or in the presence of nictonic acid was desirable. Naturally, the method, to be selective, must be based upon a reaction of the functional group and not of the pyridine ring. The Hofmann rearrangement of nicotinamide to yield 3-aminopyridine which according to the literature reacted like a normal aromatic amine and could be diazotized and coupled to yield true azo dyes appeared to offer the possibility of a direct method for its colorimetric determination (10, 11, 15, 16, 17, 18, 19, 20). The method to be described is based upon this series of reactions wherein diazotized 3-aminopyridine is coupled with N-(1-naphthyl)ethylenediamine to produce a red dye. This dye exhibits an absorption maximum at 500 mμ (Fig. 1).

In order to ascertain the feasibility of the method, it was first necessary to establish that the dye produced by this coupling reaction gave absorption values which conformed to Beer's law. Purified 3-aminopyridine was prepared from nicotinamide by the method of Camps (11). An aqueous solution was prepared and definite amounts of the amine were diazotized and coupled with N-(1-naphthyl)ethylenediamine by the procedure described in the experimental portion. The per cent transmittances at 500 mμ wave length were then determined and the results of a typical series have been recorded graphically in Fig. 2 in which extinction values (log I_0/I) are plotted against concentration.

After it had been proved that the dye produced in the coupling reaction exhibited an absorption which conformed to Beer's law, it was next necessary to determine whether a linear relationship would be established between the quantity of nicotinamide used in the Hofmann rearrangement and the absorption displayed by the dye obtained after coupling. It is generally recognized that the Hofmann rearrangement does not give 100% conversion because of the occurrence of some hydrolysis; therefore, since we now are plotting the concentration of amide instead of the amino compound against absorption, the equation combining Lambert's and Beer's laws must be written,

$$E = \log I_0/I = k(c - x)l$$

It was assumed that, if the experimental conditions could be properly controlled, the value of x would be determined by the relative concentrations of the amide, hypohalite and hydroxide in the reaction mixture and that, despite the fact that 100% conversion might not be obtained, there should be a region over which the value of x was relatively constant. The general absorption equation would then be written,

$$E = \log I_0/I = k(c - a)l$$

That this assumption was essentially correct is obvious from Fig. 3 in which the results of a typical series of determinations have been graphically recorded.

The selection of the coupling agent was based upon the work of Bratton and Marshall who determined that N-(1-naphthyl)-ethylenediamine displayed rapidity of coupling, sensitivity and reproducibility, and was unaffected by *p*H changes from 1 to 2. Furthermore, it can be prepared in a high state of purity and the azo dye produced with sulfanilamide was acid soluble (21). The maximum color intensity with this reagent is obtained in less than two minutes and remains constant in subdued light at room temperature for more than two hours thus allowing one considerable time to determine the extinction value. If exposed to sunlight for about twelve hours, the color gradually changes to yellow which may be converted to red by making the solution alkaline. This is evidently due to the hydrolysis of the N-1-naphthyl linkage in acid solution to form alpha-naphthol because the spectrophotometric absorption curve now appears to be identical with that of the dye obtained by coupling diazotized 3-aminopyridine with alpha-naphthol in basic solution.

Several other coupling agents have been investigated and the results are recorded in Table I. Alpha-napthol may be used as an appropriate coupling agent in basic solution.

Nicotinic acid, even if present to the extent of 50% of the 0.175-mg. sample, does not interfere with the determinations. Interference due to the other vitamins, when present in the amounts normally used in vitamin mixtures, has been encountered only with ascorbic acid. This is due to the ease of oxidation of the latter, thereby utilizing the hypobromite. Complex vitamin preparations containing yeast and liver extracts cannot be assayed by this method because of the presence of interfering substances, but the method can be used to detect nicotinamide in such preparations.

The method may be modified to conform to ordinary chemical colorimetry with a certain loss of sensitivity. In employing this technique it must be remembered that we are making comparisons of the total visible light transmitted, most of which is derived from those spectral regions where the solution absorbs the least light; consequently the percentage change in light intensity must always be less than whenever

measurements are made with light of a wave length corresponding to the maximum absorption band.

As with other chemical methods, this method is open to criticism because it is not entirely specific and involves a series of reactions which permits the introduction of errors. Any amide of an aromatic carboxylic acid which may undergo the Hofmann rearrangement to yield a diazotizable amine would, of course, couple to produce a dye. An aromatic amine, if present in sufficient concentration, would also affect the determination; however, in the preparation of the sample for analysis it will be observed that a considerable dilution is made so that the influence of interfering substances present in synthetic vitamin preparations is rendered practically negligible.

It is true that the Hofmann rearrangement is subject to a number of variations if not carefully controlled. The presence of an excess of hypohalite would not appear to introduce any complications since it is not probable that the pyridine ring would be ruptured to produce a cyanide. Some of the 3-aminopyridine first formed may react with unchanged isocyanate to give a bisurea (RNH—CO—NHR) and possibly some bromination of the pyridine ring is effected, but these reactions should also occur with the known samples of nicotinamide used to prepare the transmittance-concentration graph. It is believed that in this method most of these side reactions have been avoided and that yields of the amine from nicotinamide approaching the theoretical are obtained. However, it is important that the relative concentrations of the amide and reagents be maintained within the limits suggested in order to obtain valid results.

TABLE I.—AZO DYES PREPARED FROM DIAZOTIZED 3-AMINOPYRIDINE

Coupling Agent	Reaction of Coupling Solution	Color of a 3-Mg. % Solution of the Dye	
		Acidic	Basic
Alpha-naphthylamine	Acidic	Orange	Orange-red
Resorcinol	Basic	Yellow	Orange-red
Beta-naphthol	Basic	Yellow	Orange-red
Alpha-naphthol	Basic	Yellow	Red
1-Amino-8-hydroxy-3,6-naphthalene disulfonic acid	Acidic	Red	Purple-red
Phenol	Basic	Slightly yellow	Yellow

EXPERIMENTAL

Reagents.—1. A stock solution containing 1 mg. of nicotinamide per cc. This solution should be stored in the refrigerator in a brown glass bottle. Since an appreciable quantity of nicotinamide may be utilized by the microörganisms which contaminate the distilled water (3), it is recommended that a fresh solution be prepared every two weeks.

2. Potassium hypobromite solution contains in each cc. approximately 0.7 mg. of bromine and 3.00 mg. of potassium hydroxide. The presence of carbonate in the hydroxide is desirable since Chichibabin has demonstrated that the presence of carbon dioxide favors the coupling of diazotized aminopyridines with aromatic amines (22). This solution may be prepared by dissolving 0.3 Gm. of potassium hydroxide U. S. P. in about 50 cc. of distilled water. To this is added 2.80 cc. of bromine water prepared by adding an excess of bromine to distilled water and storing the mixture in a refrigerator at about 5° C. until the water is saturated. Sufficient distilled water is then added to bring the volume to 100 cc. This solution should be stored in the refrigerator in a Pyrex glass bottle stoppered with a paraffin-impregnated cork. It is recommended that this solution be prepared fresh every two weeks.

3. 1.25 *N* sulfuric acid.
4. Alcohol.
5. Sodium nitrite solution, 0.1%.
6. Ammonium sulfamate solution, 0.5%.
7. N-(1-naphthyl)ethylenediamine hydrochloride solution containing 3 mg. of the coupling agent per cc. This solution should be stored in the refrigerator in a brown glass bottle.

Preparation of Samples.—An appropriate quantity, accurately measured, of an aqueous solution of nicotinamide is transferred to a volumetric flask and diluted with sufficient distilled water to bring the amide concentration to 0.175 mg. per cc. If the solution is hydroalcoholic, such as an elixir, an appropriate quantity is transferred to a volumetric flask, the alcohol is removed under reduced pressure and the residual liquid is then diluted with distilled water as described above. In the case of synthetic vitamin mixtures, tablets and capsules a representative sample (accurately weighed if a powder) is placed in a volumetric flask, some distilled water added and the mixture warmed slightly on a water bath. The flask is shaken until the material has dissolved or is uniformly dispersed. The addition of about 1 cc. of chloroform will aid in dispersing the oleaginous vitamin preparations. Sufficient distilled water is then added so that the liquid contains 0.175 mg. of nicotinamide per cc. The liquid is filtered and the first portion of the filtrate is discarded to avoid error due to adsorption of nicotinamide by the filter paper. If ascorbic acid is present in the synthetic vitamin mixture, the representative sample is placed in a volumetric flask and dissolved or dispersed in distilled water as described. A 2% potassium permanganate solution is then added dropwise to the mixture until the pink coloration produced by the last drop of permanganate solution persists for a short time after swirling the flask. The sample is then diluted to the required volume with distilled water, filtered, and the filtrate is collected as described. If the determination is not made immediately, the prepared samples should be stored in the refrigerator.

Procedure.—Place 1 cc. of potassium hypobromite solution in each of several clean test tubes and heat them in a water bath maintained at 70° C. When the hypobromite solution has been heated, add 1 cc. of solutions prepared from the stock nicotinamide solution in such manner that they contain exactly 0.1, 0.15, 0.20, 0.25 and 0.30 mg. each per cc. of nicotinamide to test tubes numbered 1, 2, 3, 4 and 5, respectively. To the other tubes, appropriately

labeled, add exactly 1 cc. of the sample solutions prepared for analysis. Mix the contents of all tubes thoroughly by shaking and continue to heat at 70° C. for thirty minutes. At the end of this period, remove all the test tubes from the waterbath and allow to cool to about 50° C. Add 0.2 cc. (4 drops) of alcohol, allow to stand for five minutes and then add 1 cc. of 1.25 *N* sulfuric acid to each tube.

Chill the contents of the tubes by placing them in a salt-ice bath to bring the temperature to 0–5° C. When cold, add 1 cc. of cold sodium nitrite solution to each, mix thoroughly, wash down the inner wall with a little distilled water and allow to stand for three minutes in the salt-ice bath. Then add 1 cc. of ammonium sulfamate solution, mix thoroughly, and allow to stand for two minutes. Remove the tubes from the salt-ice bath, add 1 cc. of 0.3% N-(1-naphthyl)ethylenediamine dihydrochloride solution, mix thoroughly and wash down the wall of the test tube with sufficient distilled water to bring the volume of the liquid in each to about 10 cc. Allow to stand for about two minutes, then transfer the contents of each tube quantitatively to a 100-cc. volumetric flask. Wash each tube with fresh portions of distilled water, add the washings to the respective flask and finally add sufficient distilled water to bring the volume of each to exactly 100 cc. Mix thoroughly and determine the extinction value of each at 500 mμ wave length. If the samples of nicotinamide solutions prepared for analysis are colored, it is necessary to prepare a blank, omitting the coupling agent, before making the final dilution. The true absorption value of the coupled dye can then be obtained by subtracting the absorption of the blank from the absorption of the respective sample. Distilled water may be used as a satisfactory blank for colorless solutions.

Analysis of Results.—In using an instrument such as the Coleman D. M. spectrophotometer, the measurements are made as per cent transmittance which may be plotted as ordinates on semilogarithmic paper against the concentrations of nicotinamide as abscissas. The values obtained by measurement of the transmittances of tubes numbered 1, 2, 3, 4 and 5 are used to plot the linear relationship between transmittance and nicotinamide concentration. The concentration of nicotinamide in an unknown sample may be obtained by interpolation from this working graph after the transmittance of the sample has been determined and the absolute quantity of nicotinamide in the original material may be calculated by multiplying this value by the appropriate dilution factor.

It has been found that this linear relationship is valid for final concentrations of nicotinamide corresponding to from 0.75 to 3.25 micrograms per cc. (equivalent to 0.075 to 0.325 mg. of the amide used in the Hofmann rearrangement). The measurements of transmittance on known samples of nicotinamide can be duplicated, using the standardized technique, with a precision of ±2%. After the working graph has been prepared, it may be used for subsequent determinations; however, it is recommended that one or two known samples be

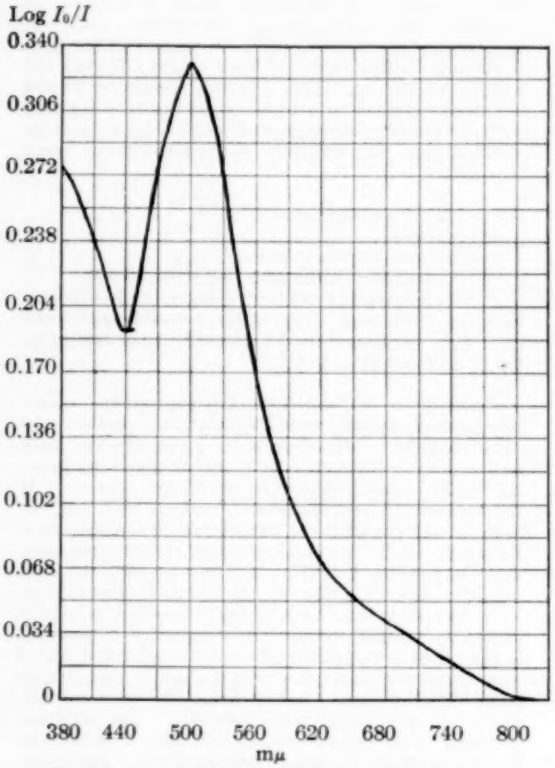

Fig. 1.—Spectrophotometric absorption curve of the dye obtained by coupling diazotized 3-aminopyridine (1.2 γ/cc.) with N-(1-naphthyl)ethylenediamine.

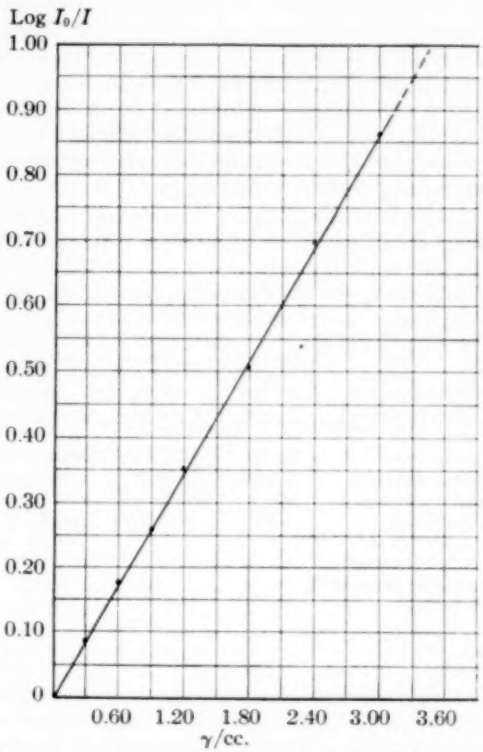

Fig. 2.—Absorption-concentration graph for the dye obtained from 3-aminopyridine at 500 mμ.

included as a check on manipulative technique. A typical working graph is reproduced in Fig. 4.

Discussion.—It is important that the Hofmann rearrangement be carefully performed in order to obtain valid results. The temperature of the water bath in which the tubes are immersed should be controlled to ±2° C. The dye obtained by coupling diazotized 3-aminopyridine with N-(1-naphthyl)-ethylenediamine possesses a large absorption coefficient; therefore small variations in the concentration of nicotinamide, provided it is effectively rearranged to the amine, effect appreciable changes in the transmittance resulting in a high sensitivity of the method.

Examination of Table I indicates that several of the diazo dyes prepared from 3-aminopyridine may be used as satisfactory indicators and that the colors are deeper than the corresponding phenyl dyes.

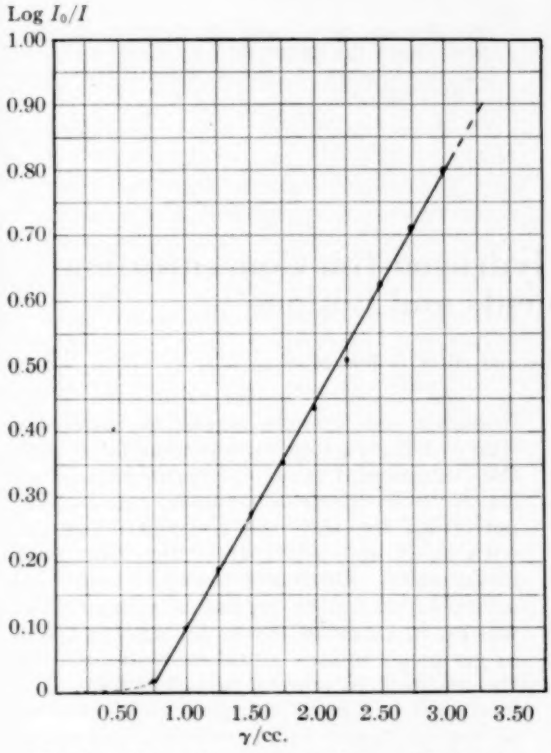

Fig. 3.—Absorption-concentration graph for the dye obtained from nicotinamide.

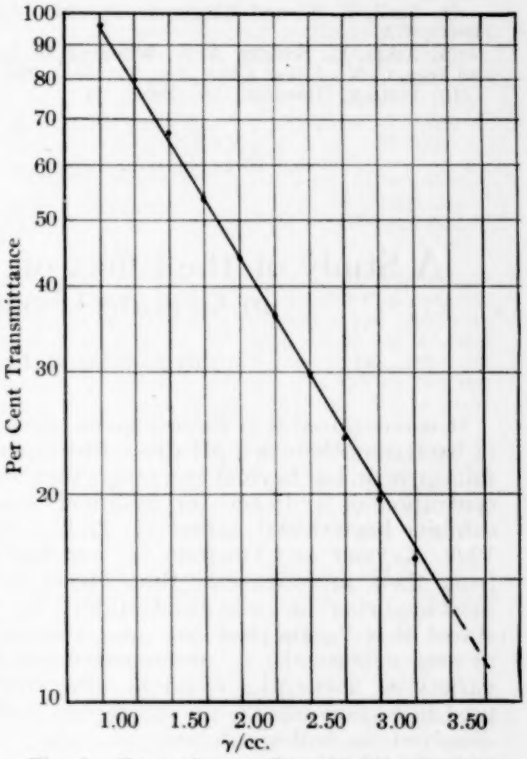

Fig. 4.—Transmittance-Concentration Working Graph for Nicotinamide.

SUMMARY

A new physiochemical method for the direct quantitative determination of nicotinamide in pharmaceutical preparations has been described which has been successfully used for the analysis of a large number of samples. The method is based upon the Hofmann rearrangement of the amide to yield 3-aminopyridine which is then diazotized and coupled with N-(1-naphthyl)-ethylenediamine to yield a red dye. The coupling occurs rapidly so that the color reaches a maximum intensity within two minutes and remains constant in subdued light at room temperature for more than two hours. The technique has been standardized so that it is possible to prepare a transmittance-concentration working graph from which the concentrations of unknown nicotinamide solutions may be interpolated after measuring their transmittances.

The presence of nicotinic acid in equivalent concentration does not interfere with the determination. This is also true of the other vitamins in the amounts in which they are ordinarily present in pharmaceutical preparations with the exception of ascorbic acid. A modification of the method which eliminates the interference caused by ascorbic acid is described.

This method possesses several distinct advantages as compared with previously reported methods, namely, specificity, convenience in the preparation of reagents, simplicity of technique and high sensitivity. The results obtained have been found to be reproducible with a precision of ±2%.

A number of diazo dyes have been prepared from diazotized 3-aminopyridine and appropriate coupling agents and their colors in acidic and basic solution have been recorded in this report.

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A Study of the Effect of Hydrogen-Ion Concentration in Certain Ointments and Lotions*

By Lorrayne E. LeMar and Allen I. White†

It is recognized that for any given species of bacterium there is a *p*H range which permits growth, but beyond this range, the concentration of hydrogen (or hydroxyl) ions exhibits bactericidal action (1, 2, 3). In 1926, Keysser and Ornstein (4) concluded from their investigations that *p*H is the most important factor in disinfection. They stated that "antiseptics are most effective at some definite *p*H. . ." and reported that a variety of antiseptics could be effectively used in certain cases if the antiseptics were dissolved in buffer solutions of optimum bactericidal *p*H.

Bittenbender, Degering and others (5, 6, 7), from the results of a series of investigations concerning the effects of *p*H on the bactericidal properties of various antiseptics, postulated a "hydrogen-ion effect," which is independent of the molecular structure of the antiseptic excluding instability or insolubility in acid media. They reported a marked increase in bactericidal effectiveness, up to a maximum, with an increase in hydrogen-ion concentration. Goedrich (8) has also made an extensive study of the various factors associated with the bactericidal action of germicides in relation to hydrogen-ion concentration. He performed his test first by controlling *p*H with acids and alkali and then later with buffer salts. He found that the combined action of an active germicide plus an acid was far in excess of the action of either acid or germicide alone. Further, he found that the addition of neutral salts to buffer salts increased still more the germicidal activity of the mixture. Lundy (9) from his study of the effect of salts upon the germicidal action of phenol and *sec*-amyltricresol, also reported that it is not through a lowering of the hydrogen-ion concentration alone that salts enhance germicidal activity but that some other factor is involved. Thus the experimental evidence points to the conclusion that when buffer mixtures are used to control the hydrogen-ion concentration of germicidal solutions, at least two factors are involved: hydrogen-ion concentration and a "salt action."

Very little work has been reported concerning the method or the effect of controlling the hydrogen-ion concentration in ointments or lotions. Goedrich (10) in 1941 reported that "an ointment containing 10% ammoniated mercury showed a clear zone of 12 mm. on an agar cup plate infected with *Staphlococcus aureus*, whereas the same concentra-

* Abstract from a thesis submitted by Lorrayne E. LeMar to the Graduate School of the State College of Washington in partial fulfillment of the requirements for the degree of Master of Science. (Presented to the Section on Practical Pharmacy of the A. PH. A., Denver meeting, 1942).

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tion of this antiseptic in the same medium showed a clear zone of 16 mm. when the *p*H was lowered by the addition of a small amount of acid." In the report, no indication was given of the *p*H of the second ointment after the addition of the acid. Pillsbury and Shaffer (11) prepared buffered "ointments" by incorporating four parts by weight of aminoacetic acid–HCl (*p*H of 2) or aminoacetic acid–NaOH (*p*H of 9.6) in one part of cholesterolized petrolatum and tested the resulting cream on a representative variety of common dermatological conditions. No evidence was given in the report that the final *p*H of the cholesterolized petrolatum–buffer mixture was determined. They did report, however, that neither the acid nor the alkali affected adversely the various dermatoses. Zakarias (12) has reported the buffering of "Physiol" colloid ointments at a *p*H of 6.9. No mention was made of how the buffering was accomplished, how the *p*H was measured, or if the *p*H had a direct bearing on the germicidal effect of the ointments.

After the completion of the work reported in this paper, there arrived in this country the publication by Harry (13) in which he recommended the use of buffered creams to correct abnormal *p*H caused by diseases of the skin. Also, he pointed out the important role *p*H plays in the defensive mechanisms of the skin and suggested that all lotions and creams be controlled to a *p*H near that of the normal skin (average *p*H 5.4) except when necessary to correct pathological conditions. He suggested the use of citric acid and sodium phosphate as buffers in such preparations.

Since the recent introduction of emulsified ointment bases having an affinity for water has made it possible to control hydrogen-ion concentration in ointments, it seemed desirable to ascertain if an increase in germicidal efficiency of ointments and lotions would accompany a controlled increase in hydrogen-ion concentration.

EXPERIMENTAL

Ointment Bases.—It was recognized that to determine *p*H of an emulsified ointment or lotion, the external phase must be aqueous in order to serve as a conductor. Moreover, as shown by Wood (14), the oil-in-water type of emulsified ointments exhibits greater bactericidal action than the water-in-oil type. Various ointment bases (15, 16, 17) were tested for their suitability to this problem. However, it was found necessary to modify these bases to make them suitable for the experimental work. For the sake of comparison two different types of hydrophilic bases were used. The following preparation is termed in this paper "Cetyl Alcohol Base." When prepared with buffers of low *p*H, it was occasionally attacked by molds. If left in the open air, the product dries out very quickly; otherwise it shelf tested very well.

Sodium Lauryl Sulfate	0.6 Gm.
Cetyl Alcohol	12.0 Gm.
White Wax	9.0 Gm.
White Petrolatum	30.0 Gm.
Buffer (or distilled water)	75.0 cc.

The white wax, petrolatum and cetyl alcohol were melted on a water bath. The sodium lauryl sulfate (and the medicament in the case of the "emulsified" ointments) was dissolved in the buffer solution and warmed to the same temperature as the wax mixture. The aqueous solution was then added to the wax mixture (or vice versa) and stirred until cool.

The second base used had the following formula and is termed in this paper "Sodium Alginate Base."

Sodium Alginate	1.0 Gm.
Buffer (or distilled water)	30.0 cc.
Sulfonated Hydrogenated Castor Oil	4.0 Gm.
White Petrolatum	15.0 Gm.
White Wax	1.0 Gm

The buffer (or water) was added to the sodium alginate and allowed to stand until a clear, smooth jelly resulted. The wax, petrolatum and sulfonated hydrogenated castor oil were melted on a water bath. The jelly was then heated to the same temperature as the wax mixture and the melted fats added to the sodium alginate jelly. The mixture was stirred until cool.

Lotion Base.—A published formula (18) was used to manufacture the lotion base. It did exhibit several incompatibilities. It will tolerate 1% phenol but the emulsion is split by a 2% concentration. Juniper tar and salicylic acid also break the emulsion.

Manufacture of Medicinal Ointments and Lotions.—The medicinal ointments prepared with the bases described above were made by mechanically incorporating the medicinal agents into previously prepared bases except in those cases where the medicaments are termed "emulsified." In these instances, the medicinal agent was dissolved in the buffer–emulsifying solution before the base was manufactured. All lotions were prepared by analogous methods.

The substances and per cent of each used in ointments in this investigation were: U. S. P. boric acid, 5%; U. S. P. calomel, 30%; U. S. P. ammoniated mercury, 5% and 10%; sulfathiazole,[1] 5% and 10%; and Roccal,[2] 1%. The substances and per cent of each used to prepare lotions were: U. S. P. phenol, 1%; rotenone, 2%; and Roccal, 1%.

Control of Hydrogen-Ion Concentration in Ointments and Lotions; Buffer Solutions.—Clark and Lubs' buffer mixtures were prepared as directed by the U. S. P. XI (19). McIlvaine buffer solutions were prepared according to the directions of Clark (20). Five drops of methyl salicylate per liter were added to the stock solutions of citric acid

[1] The authors are indebted to Merck and Co. for supplying the sulfathiazole used in this investigation.

[2] Roccal is a patent product of the Alba Pharmaceutical Company and consists of a high molecular group of alkyldimethylbenzylammonium chlorides.

and potassium acid phosphate to prevent the growth of mold. The range of *p*H of the first two series of buffer solutions was from 4 to 7 with an interval of 0.4 of a *p*H. The range of *p*H of the third series of buffer solutions was from 3 to 8 with an interval of 1 *p*H unit. The hydrogen-ion concentration of the buffer solutions was determined electrometrically using a quinhydrone electrode and sometimes a glass electrode.

Effectiveness of Buffer Solutions.—The above-mentioned buffer solutions were used to control the hydrogen-ion concentration of the ointments and lotions by substituting the buffers for the distilled water called for in the formulas of the preparations. Tables I, II and III indicate the effectiveness of the buffer solutions in controlling the *p*H of the ointments and potassium acid phosphate to prevent the growth of mold. The range of *p*H of the first two series of buffer solutions was from 4 to 7 with an interval of 0.4 of a *p*H. The range of *p*H of the third series of buffer solutions was from 3 to 8 with an interval of 1 *p*H unit. The hydrogen-ion concentration of the buffer solutions was determined electrometrically using a quinhydrone electrode and sometimes a glass electrode.

Effectiveness of Buffer Solutions.—The above-mentioned buffer solutions were used to control the hydrogen-ion concentration of the ointments and lotions by substituting the buffers for the distilled water called for in the formulas of the preparations. Tables I, II and III indicate the effectiveness of the buffer solutions in controlling the *p*H of the ointments and lotions. Tests were made to determine whether or not the emulsifying agents in the formulas were responsible for the change in *p*H. Buffer solutions to which sodium lauryl sulfate or sodium alginate had been added in the same concentration as used in the formulas showed little change in *p*H. (a change of *p*H 0.2). However, when the other ingredients of the bases were added, a marked change usually resulted. Incorporation of medicaments changed the *p*H still more.

Methods Used to Incorporate Medicaments.—In the manufacture of the medicated ointments two methods of approach were possible: the medicament might be dissolved in either the buffer-emulsifying agent solution or the wax-fat mixture depending upon its solubility, and then the ointment prepared in the usual manner (referred to in the tables as "emulsification"); or the medicament might be mechanically incorporated after the base was compounded. In order to ascertain if the method of incorporating the medicament would have an effect upon the resulting *p*H of the completed ointment, a total of 26 ointments were made up by each method and the *p*H of each determined. In no case was the difference in *p*H significant.

Methods of Measuring Hydrogen-Ion Concentration in Ointments and Lotions.—Two methods were used to test the hydrogen-ion concentration in the ointments and lotions. The first method employed a bulb-type glass electrode and a Beckman Industrial Model *p*H meter in the usual manner. However, because of the well-known poisonous action of fats on glass electrodes, another method was investigated. This method is an adaptation of Golding's[3] modification of the method used by Watson (21, 22) to test the *p*H of certain types of cheese which contain free fat. This second method was studied using both a Beckman *p*H meter and a Leeds and Northrup Type K potentiometer as the measuring instruments. Descriptions of the methods follow.

Quinhydrone with Beckman pH Meter.—One to two Gm. of ointment was triturated in a mortar or spatulated on a pill tile with a small amount of quinhydrone and the ointment-quinhydrone mixture was then filled into a one-inch length of soda straw. Into this was inserted a flame-cleaned gold wire which had been checked previously against a standard phthalate buffer (23). Care must be taken not to allow the tip of the electrode to protrude through the lower surface of the ointment in the straw but placed close enough to the surface to insure completion of the circuit. The free end of the wire was attached to the *p*H meter by means of a suitable battery clip. The calomel electrode and the gold wire bearing the ointment were dipped into a beaker containing a saturated solution of KCl and calomel and the *p*H of the ointment then read directly from the

TABLE I.—EFFECT OF BUFFERS ON *p*H OF OINTMENTS IN CETYL ALCOHOL BASE

Buffer	*p*H of Buffer	*p*H of Base	*p*H of Ointments: Sulfathiazole 5%	Sulfathiazole 10%	Boric Acid 5%	Calomel 30%	Ammoniated Mercury 10%	Roccal 1% Emul.[a]	Roccal 1% Mech.[b]
McIlvaine									
1	4.09	4.03	4.10	4.10	3.82	4.02	3.92	..	..
2	4.50	4.48	4.47	4.44	4.10	4.43	3.92	..	..
3	4.83	4.85	4.81	4.87	4.49	4.76	4.45	..	..
4	5.24	5.24	5.18	5.22	4.73	5.12	4.19	..	..
5	5.66	5.50	5.52	5.52	5.01	5.47	4.43	..	..
6	6.10	5.84	5.83	5.89	5.30	5.83	5.83	..	..
7	6.45	6.18	6.17	6.19	5.49	6.14	6.03	..	..
8	6.92	6.48	..	6.53	5.62	6.50	6.42	..	..
9	7.23	6.77	6.82	6.87	5.72	6.75	6.70	..	..
10	3.12	3.58	..	..	..	..	3.61	3.04	3.03
11	4.00	4.49	..	..	..	..	3.79	3.87	3.95
12	4.99	5.09	..	..	.	..	4.28	4.90	4.86
13	5.97	6.11	..	..	..	..	5.10	5.71	5.62
14	6.87	6.62	..	..	..	..	6.60	6.62	6.50
15	7.38	6.79	..	..	..	..	6.91	7.18	7.18
Clark and Lubs									
1	3.45	4.09	..	..	..	..	4.21	3.53	3.54
2	3.86	4.21	..	..	..	..	4.21	4.10	4.05
5	4.92	4.59	..	..	..	..	4.71	4.92	5.00
4	5.84	5.32	..	..	..	..	6.05	5.63	5.62
5	7.06	6.02	..	..	..	..	6.50	6.30	6.48
6	7.42	6.36	..	..	..	..	6.63	6.62	6.68
None	..	4.67	4.84	4.89	4.25	4.62	4.50	5.10	5.70

[a] Emulsified.
[b] Mechanically incorporated.

[3] Dr. N. S. Golding, Assoc. Prof. of Dairy Husbandry, State College of Washington.

*p*H meter. The gold wires were washed with distilled water and flamed between each test.

Determinations with a Potentiometer.—A Leeds and Northrup Type K potentiometer was used in this method. The quinhydrone electrode consisted of a gold wire coiled around a glass tube. Eight to 10 Gm. of ointment or lotion were mixed with quinhydrone and allowed to stand until an equilibrium was reached. The calomel and gold electrodes were then placed in the ointment (or lotion)-quinhydrone mixture. The e. m. f. of the cell was then read and the *p*H calculated.

corporation of the quinhydrone into the ointment or lotion and the reading of the *p*H would have upon the results obtained. A study on twelve ointments and six lotions of varying *p*H showed that in an acid *p*H, only a very slight drift(toward alkalinity) was noticeable when readings were made within one minute after incorporating the quinhydrone, at ten minutes, fifteen minutes and sixty minutes. However, as the *p*H approached alkalinity, the drift (toward acidity) became more marked. For this reason, in all preparations having a *p*H of 5.5 or more, the readings were taken within

TABLE II.—EFFECT OF BUFFERS ON *p*H OF OINTMENTS IN SODIUM ALGINATE BASE

Buffer	*p*H of buffer	*p*H of Base	*p*H of Ointments: Ammoniated Mercury 10%	Ammoniated Mercury 5%	Roccal 1% Emul.[a]	Roccal 1% Mech.[b]
McIlvaine						
10	3.12	3.89	2.18	..	3.62	3.51
11	4.00	4.31	2.70	..	4.56	4.40
12	5.09	5.13	3.30	..	5.22	5.22
13	5.97	5.75	3.86	..	5.78	5.81
14	6.87	6.40	4.61	..	6.33	6.49
15	7.36	6.80	5.00	..	6.88	6.82
Clark and Lubs						
1	3.45	4.61	3.30	3.08	4.62	4.61
2	3.86	4.93	3.43	3.52	5.07	5.00
3	4.92	5.40	3.81	3.58	5.41	5.71
4	5.84	5.87	4.51	4.25	5.98	5.97
5	7.06	6.29	4.31	4.60	6.39	6.30
6	7.42	6.45	4.50	4.73	6.43	6.50
None		6.30	4.02	4.30	6.04	6.02

[a] Emulsified.
[b] Mechanically incorporated.

TABLE III.—EFFECT OF BUFFERS ON *p*H OF LOTIONS

Lotion	*p*H of Buffer	*p*H of Lotion
Phenol 1%		
Mech.[a]	4.89	4.81
Emul.[b]	4.89	4.86
Rotenone 2%		
1	4.09	4.18
2	4.50	4.35
3	4.83	4.49
4	5.24	4.71
5	5.66	5.18
6	6.10	5.36
7	6.45	5.59
8	6.92	6.40
9	7.23	6.97
No buffer		6.92

[a] Mechanically incorporated.
[b] Emulsified.

TABLE IV.—COMPARISON OF *p*H READINGS OBTAINED BY VARIOUS METHODS

Series	Material Tested	Method: Glass Electrode	Quinhydrone: Beckman *p*H Meter	Quinhydrone: Potentiometer
1	Buffer	2.97	2.96	..
	Ointment base	2.88	2.92	..
	Roccal 1%	2.88	2.88	..
	Ammon. merc. 5%	5.28	2.75	..
2	Buffer	3.88	3.88	..
	Ointment base	3.80	3.85	..
	Roccal 1%	3.84	3.88	..
	Ammon. merc. 5%	5.65	3.48	..
3.	Buffer	4.87	4.86	..
	Ointment base	4.78	4.70	..
	Roccal 1%	4.79	4.81	..
	Ammon. merc. 5%	5.92	4.18	..
4	Buffer	5.89	5.86	..
	Ointment base	5.62	5.62	..
	Roccal 1%	5.66	5.69	..
	Ammon. merc. 5%	6.15	5.89	..
5	Buffer	6.85	6.80	..
	Ointment base	6.42	6.45	..
	Roccal 1%	6.46	6.50	..
	Ammon. merc. 5%	6.61	6.45	..
6	Buffer	7.85	7.79	..
	Ointment base	7.00	7.00	..
	Roccal 1%	7.00	7.08	..
	Ammon. merc. 5%	7.08	6.96	..
7	Unbuffered[a]	..	..	..
	Ointment base	4.40	4.60	..
	Roccal 1%	4.57	4.60	..
	Ammon. merc. 5%	6.53	5.62	..
8	Phenol lotion 1%	4.85	4.85	4.82
9	McIlvaine buffers	..	4.09	4.21
			4.44	4.48
			4.88	4.83
			5.68	5.66
			6.02	6.10
			6.41	6.49
			6.48	6.45

[a] Distilled water used to prepare ointments in place of buffer solution.

Comparison of Methods.—Table IV shows the close agreement of the results obtained by the two methods. The close agreement between the results obtained with a glass electrode and the quinhydrone methods is an indication of the accuracy of the latter method of determining the hydrogen-ion concentration of ointments and lotions. The variance in the methods in the case of the 5% ammoniated mercury ointment is unexplained.

Time Element in Determining pH by Quinhydrone Method.—There was some question as to the effect that the length of time elapsing between the in-

ten minutes after the quinhydrone was mixed with the material to be tested.

Determination of Bactericidal Action; Standardization of Test Organism.—The F. D. A. strain of *Staphylococcus aureus*, obtained from the Department of Bacteriology and Public Health of the State College of Washington, used in the tests was standardized against phenol according to the F. D. A. specifications (24) with the following modifications: The nutrient broth used for the daily transfers was adjusted to *p*H 7.3 to 7.4 as this is the optimum *p*H for *S. aureus* (25); the cultures were incubated at a

temperature ranging from 34° to 36° C.; subcultures were made in a semisolid media (26). The final *p*H as determined colorimetrically was 7.4.

The 5% stock solution of phenol from which the 1:60 and 1:70 dilutions were made was prepared as follows: 5 Gm. of phenol, A. R., which had been stored in a desiccator were weighed on a prescription balance and then returned to the desiccator for removal of any absorbed moisture. An analytical balance, kept relatively free of moisture by anhydrous calcium chloride, was prepared to weigh 5 Gm. Then the phenol previously weighed on a prescription balance was adjusted to 5.000 Gm. on the analytical balance, and dissolved in sufficient sterile distilled water to make 100 cc. of solution at 20° C.

All glassware, water and materials used in the phenol test were sterilized before using. The results of the phenol tests showed that the test organism survived at 1:60 dilution of phenol for five minutes (for ten minutes in the second test) and a 1:70 dilution for fifteen minutes. The test was performed at 20° C.

Agar Cup Plate Tests.—The bactericidal activity of the ointments were tested according to the F. D. A. agar cup plate method (24) using the standardized strain of *S. aureus*. Tables V, VI and VII show the results of the agar cup plate tests on medicated ointments having varying hydrogen-ion concentrations. In general, the lower the *p*H, the wider the zone of inhibition. In the case of the sulfathiazole ointments, although the width of the zones was approximately the same, there was a noticeable difference in the clarity of the zone and the amount of growth obtained in the subcultures. The effect of *p*H on the bactericidal action of 1% Roccal ointments in both the cetyl alcohol and sodium alginate bases was also tested. The unbuffered ointments failed to show any bactericidal or bacteriostatic action and while the lowering of *p*H by use of buffers did produce clear zones in agar cup plates up to 5 mm., the results were too indefinite to be interpreted.

Determination of Fungicidal Action; Standardization of Test Organisms.—The fungi, *Trichophyton interdigitale* and *Trichophyton rosaceum*, obtained from the Department of Bacteriology and Public Health of the State College of Washington, used in the tests were standardized against phenol according to the F. D. A. specifications (24). Fourteen-day-old cultures on Sabouraud's dextrose agar of each organism were tested as follows: 10 cc. of sterile normal saline solution was added to each slant, the growth broken up with a platinum loop, the tube shaken vigorously, and the contents transferred aseptically to a sterile flask. Of the above cultures 0.5 cc. portions are added to 5 cc. dilutions of phenol in a sterile test tube and allowed to stand the specified time at 30° C. Subcultures were then made on Sabouraud's dextrose agar slants and then incubated at 30° C. for seven days. The *T. interdigitale* survived a 1:100 dilution for ten minutes but not for fifteen minutes. The *T. rosaceum* survived a 1:100 dilution for five minutes but not for ten minutes.

Agar Cup Plate Tests.—The fungicidal activity of the ointments and lotions were tested according to the F. D. A. agar cup plate method (24) using the standardized strains of *T. interdigitale* and *T. rosaceum*. Table VIII shows the effect of *p*H on the fungicidal action of 5% ammoniated mercury ointments. The effect of *p*H on the actions of 1% Roccal ointments and 2% rotenone and 1% Roccal lotions was also tested. In these tests clear zones of relatively greater sizes were obtained as the *p*H of the preparations decreased when tested against *T. interdigitale*. In the tests against *T. rosaceum* the sizes of the cloudy zones increased as the *p*H increased. This is an effect opposite to that obtained with the 5% ammoniated mercury ointments against the same organism.

TABLE V.—EFFECT OF *p*H ON BACTERICIDAL ACTION OF AMMONIATED MERCURY OINTMENTS

Ointment	Base: Cetyl Alcohol *p*H[a]	Cetyl Alcohol Zone, Mm.[b]	Sodium Alginate *p*H[a]	Sodium Alginate Zone, Mm.[b]
Ammoniated mercury 10%, McIlvaine buffers	3.61	11.5	2.18	16.6
	3.79	11.0	2.70	14.7
	4.28	10.0	3.30	13.7
	5.10	10.3	3.86	12.8
	6.60	8.7	4.61	10.6
	6.91	7.2	5.00	9.5
Unbuffered	4.61	8.6	4.02	9.8
Ammoniated mercury 10%, Clark and Lubs buffers	4.21	11.1	3.30	12.7
	4.21	10.1	3.43	12.0
	4.71	9.1	3.81	10.6
	6.05	9.6	4.51	10.0
	6.50	9.5	4.31	8.6
	6.63	9.0	4.50	7.8
Unbuffered	4.61	8.6	4.02	9.8
Ammoniated mercury 5%, Clark and Lubs buffers	..	..	3.08	14.7
	..	..	3.52	13.8
	..	..	3.58	12.1
	..	..	4.25	11.0
	..	..	4.60	10.3
	..	..	4.75	11.4
Unbuffered	..	..	4.30	11.4

[a] *p*H tested on Beckman *p*H meter with gold electrode.
[b] No growth was obtained in subcultures.

DISCUSSION

In choosing the ointment and lotion bases used in this study, no attempt was made to compare the many hydrophylic bases described in the recent literature. The choices were made according to simplicity of formula, ease of manufacture, and adaptability to the problem. It is probable that a study of other ointment bases would find one in which the hydrogen-ion concentration would be easier to control than in the bases used. A comparison of Table I with Table II indicates that the cetyl alcohol base is somewhat easier to buffer to the desired *p*H than is the sodium alginate base.

The buffer solutions used were adopted because they gave the desired range in hydrogen-ion concentration and are extensively used. A study of Tables I and II shows that the McIlvaine buffer solutions were slightly more efficient in these experiments than the Clark and Lubs buffer solutions. However, these tables also show that these buffer solutions are not sufficiently effective for the *p*H of the completed ointment or lotion to be predicted from the *p*H of the buffer solution. Furthermore, it has been difficult to obtain buffered ointments with regular intervals in *p*H over the wide range desirable for bactericidal study. For this reason, it might be desirable to use protein buffers in place of the inorganic salts.

At first it was believed possible that the glass electrode could be used advantageously for determining the hydrogen-ion concentrations. However, the probability that small amounts of unemulsified fats might be present in the aqueous phase made it seem desirable to seek some other method of determining *p*H. For this reason, the quinhydrone method which had already been used in determining the *p*H in cheeses was the method of choice. Although the quinhydrone method was successful, it is probable that thoroughly emulsified bases might be tested with a glass electrode without danger of "poisoning" the electrode. This would be particularly valuable for determining the hydrogen-ion concentration of those ointments approaching or in an alkaline range. It is well known that quinhydrone is inaccurate in alkaline media, and consequently, the figures representing *p*H values in the alkaline range cannot be taken as highly accurate.

The experimental work also gave evidence of some incompatibilities between the quinhydrone method of determining *p*H and the ingredients of ointments and lotions. There was a pronounced drift in the *p*H of the bases, as well as of the medicated ointments and lotions, when the mixture of ointments or lotions with quinhydrone were allowed to stand. In the case of ammoniated mercury ointments, this drift was frequently so rapid as to make an accurate reading of the *p*H difficult.

The results of the bactericidal and fungicidal tests indicate that, as in other antiseptic or germicidal agents, it may be desirable to control the hydrogen-ion concentration in hydrophilic ointments and lotions intended to be used as antiseptics and germicides. In general, the greater the hydrogen-ion concentration, the more effective the medicated ointments or lotions appeared in agar cup plate tests on *S. aureus*, *T. interdigitale* and *T. rosaceum*. Figure 1 illustrates graphically this point as found on tests with ammoniated mercury ointments. Tests on buffered ointment bases containing no medicament at various *p*H ranges showed

TABLE VI.—EFFECT OF *p*H ON BACTERICIDAL ACTION OF SULFATHIAZOLE OINTMENTS[a]

Ointment	*p*H[b]	Zone, Mm.	Appearance of Zone	Subcultures
Sulfathiazole 5% McIlvaine				
1	4.10	6.0	Clear	Slight growth
2	4.47	5.0	Clear	Slight growth
3	4.81	4.5	Clear	Moderate growth
4	5.18	5.5	Clear	Moderate growth
5	5.52	6.5	Cloudy	Moderate growth
6	5.83	6.0	Cloudy	Moderate growth
7	6.17	5.0	Very cloudy	Definite growth
8	6.82	5.0	Very cloudy	Definite growth
Unbuffered	4.84	None		
Sulfathiazole 10% McIlvaine				
1	4.10	5.0	Clear	Moderate growth
2	4.44	4.7	Clear	Moderate growth
3	4.87	5.0	Almost clear	Definite growth
4	5.22	3.0	Cloudy	Luxuriant growth
5	5.52	5.0	Cloudy	Luxuriant growth
6	5.89	4.5	Very cloudy	Luxuriant growth
7	6.19	4.5	Very cloudy	Luxuriant growth
8	6.53	4.5	Very cloudy	Luxuriant growth
Unbuffered	4.89	5.0	Cloudy	Luxuriant growth

[a] Cetyl alcohol base used exclusively.
[b] *p*H tested on Beckman *p*H meter with gold electrode.

TABLE VII.—EFFECT OF *p*H ON BACTERICIDAL ACTION OF 30% CALOMEL OINTMENTS[a]

Ointment	*p*H[b]	Zone, Mm.[c]
1	4.43	5.5
2	4.76	4.5
3	5.12	4.5
4	5.47	4.0
5	5.83	4.5
6	6.14	5.5
7	6.50	3.5
8	6.75	3.5
Unbuffered	4.62	6.0

[a] McIlvaine buffers in cetyl alcohol base used exclusively.
[b] *p*H tested on Beckman *p*H meter with gold electrode.
[c] No growth in subcultures.

that the buffered bases themselves possessed no bactericidal or fungicidal action. Thus, increased hydrogen-ion concentration is not of itself bactericidal or fungicidal in the ranges tested but apparently simply increases the efficiency of the bactericidal agent. It is also interesting to note that in most cases there is a noticeable difference in efficiency between buffered and unbuffered ointments at similar *p*H. Thus it might be suggested that the addition of salts has an effect apart from hydrogen-ion concentration.

Although it was not made a point of study for this paper, the sodium alginate base appeared to be a more effective medium than the cetyl alcohol base. It is also interesting to note that in comparing the two buffer solutions used, one did not influence the bactericidal action of an ointment more than the other when the resulting ointments had similar hydrogen-ion concentrations.

TABLE VIII.—EFFECT OF *p*H ON FUNGICIDAL ACTION OF 5% AMMONIATED MERCURY OINTMENTS.[a]

Organism	*p*H[b]	Zone, Mm.	Appearance of Zone
Trichophyton interdigitale	2.75	15.2	Clear
	3.48	10.1	Clear
	4.18	8.8	Clear
	5.89	6.5	Clear
	6.45	5.3	Clear
	6.96	4.4	Clear
	5.62[c]	7.0	Clear
Trichophyton rosaceum	2.75	13.7	Clear
	3.48	8.5	Clear
	4.18	7.3	Clear
	5.89	6.0	Clear
	6.45	4.5	Clear
	6.96	4.0	Clear
	5.62[c]	8.2	Clear

[a] McIlvaine buffers in cetyl alcohol base used exclusively.
[b] *p*H tested on Beckman *p*H meter with gold electrode.
[c] Distilled water used in place of buffer solutions.

SUMMARY AND CONCLUSIONS

1. A new method of measuring the hydrogen-ion concentration in hydrophilic ointments and lotions is described.

2. The hydrogen-ion concentration of certain hydrophilic ointments and lotions have been controlled by the use of inorganic buffer-salt solutions although the extent of the control is not so great as is desirable.

3. It has been found that the hydrogen-ion concentration of a cetyl alcohol base is more readily controlled than that of a sodium alginate base.

4. A medicament may be either mechanically incorporated into a buffered base or mixed with the buffer-emulsifying solution in the manufacture of the base without appreciably altering the final hydrogen-ion concentration.

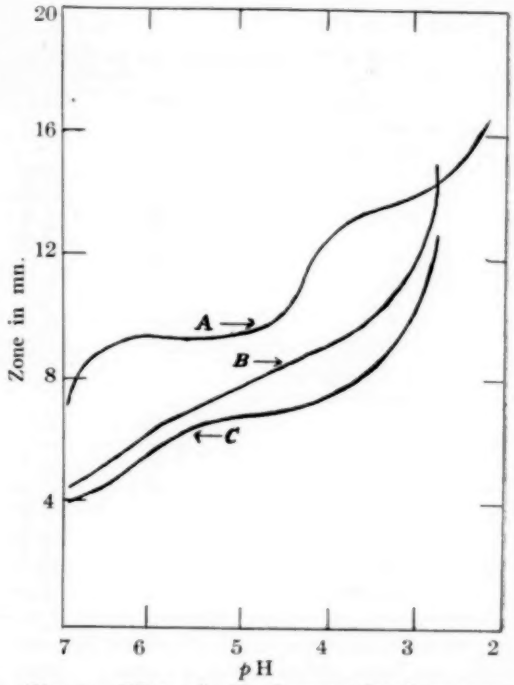

Fig. 1.—Effect of *p*H of ammoniated mercury ointments on width of zones in agar cup plates. *A*, *Staph. aureus*; *B*, *T. interdigitale*; and *C*, *T. rosaceum*.

5. Agar cup plate tests indicated that, in general, the greater the hydrogen-ion concentration, the greater the germicidal or bacteriostatic action of the preparations.

6. Certain buffered ointments tested exhibited greater germicidal action than unbuffered ointments of similar hydrogen-ion concentration.

7. The buffered ointment bases containing no added medicaments showed no bactericidal action.

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A Convenient Synthesis of β-Bromoethylphthalimide*

By Taito O. Soine†

Recently it was found necessary in this laboratory to prepare β-bromoethylphthalimide as an intermediate for the preparation of other compounds. The only method of preparation commonly used (1) is based on Gabriel's original method (2) whereby potassium phthalimide is reacted with ethylene dibromide. This method is somewhat lengthy and involves considerable manipulations. It would therefore be desirable to shorten the time required and to eliminate the lengthy purification procedure. Wenker (3) has suggested a convenient method for the preparation of β-chloroethylphthalimide which can be used in many instances in place of β-bromoethylphthalimide. However, the β-bromo compound is more reactive than the β-chloro compound and for that reason is more desirable. The following method is short, convenient, and gives good yields. It involves the initial preparation of β-hydroxyethylphthalimide by the interaction of phthalimide or phthalic anhydride with monoethanolamine. The resultant β-hydroxyethylphthalimide is then readily converted to β-bromoethylphthalimide by the action of phosphorus tribromide.

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EXPERIMENTAL

β-Hydroxyethylphthalimide.—Twenty grams of phthalimide were mixed with 8.5 Gm. of freshly distilled monoethanolamine in a 100-cc. round-bottom flask equipped with a condenser. The flask and condenser had ground glass connections. The mixture was heated for two hours on a steam bath in the hood with occasional agitation. The molten reaction mixture was then poured out while hot and allowed to crystallize, upon which an almost theoretical yield of crude material was obtained. This crystalline material was dissolved in 100 cc. of boiling distilled water, boiled for 10 minutes with decolorizing carbon (Norit), and filtered. The filtrate, on cooling in the refrigerator, deposited 20.5 Gm. of white crystals, m. p. 128–129°. The mother liquor when concentrated to a volume of about 15 cc. yielded an additional 2.5 Gm. of crystals, m. p. 128–129°. Total yield was 23 Gm. (88.5% of the theoretical). A mixed melt of these crystals with a sample prepared according to the literature (3, 4) showed no depression of the melting point.

β-Bromoethylphthalimide.—Four cubic centimeters of phosphorus tribromide was added to 10 Gm. of β-hydroxyethylphthalimide in a 100-cc., round-bottom flask fitted with a condenser having a ground glass joint. The mixture was heated for two hours in the hood on a steam bath with occasional shaking. The hot reaction mixture was poured into 100 Gm. of crushed ice with stirring. The crude β-bromoethylphthalimide, when washed with distilled water and dried, weighed 10 Gm. The mother liquor, when concentrated to a volume of 10 cc. and allowed to stand for 24 hours yielded 2 Gm. of unchanged β-hydroxyethylphthalimide. The crude β-bromoethylphthalimide was dissolved in 200 cc. of 50% alcohol, boiled for 10 minutes with decolorizing carbon (Norit), and filtered. The filtrate when cooled to 0° yielded 7.5 Gm. of white needles, m. p. 82–83°. The mother liquor yielded an additional 0.9 Gm. of product, m. p. 81–83°. The yield of purified material was 80.8% of the theoretical. A mixed melt of

this compound with a known sample of β-bromoethylphthalimide (m. p. 82–83°) showed no depression of the melting point.

SUMMARY

1. A convenient synthesis of β-bromoethylphthalimide in 80.8% yields by the bromination of β-hydroxyethylphthalimide with phosphorus tribromide is described.

2. The synthesis of β-hydroxyethylphthalimide by the interaction of phthalimide and monoethanolamine is also described.

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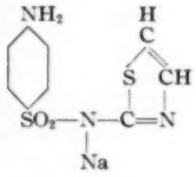
Vasosulfa Compounds*

By William F. Hamilton, Melvin F. George, Jr., Eli Simon and Frederick M. Turnbull†

A recent article by Turnbull (1) showed that beneficial results were obtained by the use of a 5% aqueous solution of sodium sulfathiazole in the treatment of chronic sinusitis. Since the publication of this information, widespread use of sodium sulfathiazole for intranasal therapy has made further development of aqueous solutions for this purpose advisable (2).

Difficulties encountered with the clinical use of aqueous sodium sulfathiazole were largely due to the instability of the solution. A precipitate formed within a relatively short period of time, and the material became discolored. The discolored solution was found irritating to the nasal mucosa (3).

Sodium sulfathiazole (sodium 2-sulfanilyl aminothiazole) may be designated structurally as:



After inspection of the structural configuration, considerable experimental work showed that the instability of the solution might be due to two main effects: (*a*) precipitation of sulfathiazole crystals after exposure to air, and (*b*) partial oxidation of the drug with the formation of complex, highly colored oxidation products.

The precipitation involves replacement of the sodium atom in the compound by hydrogen with the formation of relatively insoluble crystalline sulfathiazole. The mechanism of the oxidation is probably complex, and may be attended by the formation of such intermediates as those resulting from incomplete oxidation of the *p*-amino group on the benezene nucleus of the sulfathiazole, with the formation of the *p*-hydroxylamino or *p*-nitroso compounds (4). Assays showed that only a small proportion, less than 5%, of the total amount of sodium sulfathiazole present in the solution was affected by oxidation.

EXPERIMENTAL

In order to render aqueous solutions of sodium sulfathiazole suitable for distribution as a marketable product, stabilization is necessary. The stabilizer must simultaneously protect the drug from precipitation or from oxidation in solution, and must be acceptable for therapeutic use. Many materials for stabilizing were investigated, and only alkaline sulfites were found effective. Sodium sulfite was compatible with sodium sulfathiazole, and amounts in excess of 0.5% were effective in preventing precipitation and discoloration of the solution. To prevent rapid deterioration of the sodium sulfite, a small amount of glycerin was added to act as a secondary stabilizer (5).

Therapeutic use of an aqueous solution composed of sodium sulfathiazole sesquihydrate 2.5%, sodium sulfite anhydrous C.P. 2.0%, and glycerin 1.0% gave promising results. The mixture was light, stable upon storage, and no difficulties were encountered due to precipitation or discoloration. The clinical work showed that while the sodium sulfathiazole solution appeared to possess mild vaso-constrictive properties (1), the incorporation of a sympatho-

* Received Aug. 23, 1943, from the Research Laboratory, Chemical Division, Lockheed Aircraft Corporation, Burbank, Calif.

†The authors are indebted to the Abbott Laboratories for supplying chemicals and toxicological results included in this paper; to Eli Lilly and Co. for independent verification of one of the new compounds, and constructive suggestions; to Parke Davis & Co. for analytical work and independent syntheses verifying the existence of two of the new compounds; and to E. R. Squibb and Sons for chemicals, analytical data on two of the compounds, and valuable general guidance and advice. These companies are licensees under patents pending which cover the information disclosed in this presentation.

mimetic amine in the solution was desirable in order promptly to shrink the nasal mucosa and give relief to the patient by substantially decreasing congestion in the nasal passages.

Attempts to incorporate ephedrine alkaloid or its acid salts were unsuccessful because, sooner or later, precipitation occurred. The crystals which separated from the solution were filtered, dried and examined under the microscope. They were distinctly different in appearance from either ephedrine or sulfathiazole crystals. The melting point was found to be 201° C. (206° C. corr.). These observations indicated that compound formation had probably occurred and that the new substance formed was relatively insoluble.

Various sympathomimetic amines were investigated and some of them were found compatible with the stabilized solution. In relatively small quantities, adrenalin went into solution readily, but its use was precluded because of its undesirable side effects and its instability in storage. *dl*-Desoxyephedrine was likewise found compatible, and because of the fact that it is readily synthesized by the use of nitroparaffins (6), and is physiologically desirable, this compound was chosen for further developmental work. It was originally prepared by Ogata (7) in 1919 and, like sulfanilamide, was overlooked for 20 years.

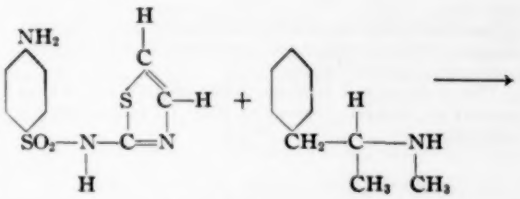
Varying amounts of *dl*-desoxyephedrine hydrochloride were added to the stabilized solution of sodium sulfathiazole, and the optimum amount of desoxyephedrine hydrochloride required for adequate pressor action of the solution was found to be 0.125%. In aqueous solutions, the clinically effective concentration of desoxyephedrine hydrochloride by itself had been determined to be in excess of 1%.(8). Thus, the effectiveness of the drug in stabilized sodium sulfathiazole solutions was approximately eight times as great as it was by itself. The precise reason underlying the "synergism" was still undetermined, and because of the fact that compound formation, like that encountered in the case of ephedrine, might account for the phenomenon, the following experiment was undertaken:

Six grams of desoxyephedrine alkaloid was dissolved in 200 cc. of distilled water containing 3 Gm. anhydrous C.P. sodium sulfite, with agitation and gentle heating. Nine grams of sulfathiazole was added with continuous stirring and heating until complete solution resulted. The mixture was then cooled in the dark and allowed to stand overnight in the refrigerator at a temperature of approximately 40° F. The crystals which formed were filtered, washed and thoroughly dried. The melting point of the product was 116° C. (uncorr.), 118° C. (corr.).

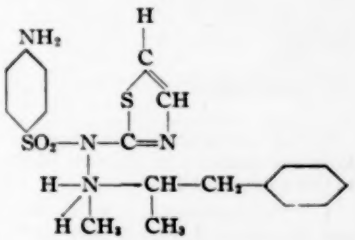
The crystals which formed in the solution were radiated columnar, monoclinic. As pure desoxyephedrine alkaloid is a colorless liquid, and as sulfathiazole crystallizes in plates which melt at a temperature of 202.5° C. (corr.), it appeared that a new chemical compound had probably been formed by this procedure.

Compound formation was not expected in the alkaline aqueous solution because the two compounds which are present in the solution are chemically somewhat similar in their behavior. However, study of the literature reveals that an amine, theophylline (1,3-dimethylxanthine), can form compounds with other amines to produce addition products such as theophylline ethylenediamine. No data were found indicating that amides form addition compounds with amines.

In the present instance, the normally trivalent nitrogen of the desoxyephedrine becomes pentavalent, and onium salt formation occurs according to the following reaction:



Sulfathiazole Desoxyephedrine



Desoxyephedronium sulfathiazole

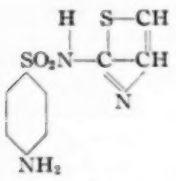
TABLE I

	Ephedronium		Desoxyephedronium	
	Sulfathiazole	Sulfadiazine	Sulfathiazole	Sulfadiazine
Empirical formula	$C_{19}H_{24}N_4S_2O_3$	$C_{20}H_{25}N_5SO_3$	$C_{19}H_{24}N_4S_2O_2$	$C_{20}H_{25}N_4SO_2$
Molecular weight	420.3	415.3	404.3	399.3
Melting point ° C.:				
Uncorrected	201	187–189	116–118	183–185
Corrected	206	192–193	118–120	187–189
Per cent nitrogen:				
Calculated	13.33	16.86	13.86	17.54
Found	13.38	17.07	14.23	17.80
Per cent sulfur:				
Calculated	15.24	7.72	15.86	8.03
Found	15.03	7.81	15.83	8.02
Solubility in 100 Gm. water, Gm.:				
At 30° C.	0.16	1.13	1.67	1.52
At 2° C.	0.11	0.77	1.19	1.25
*p*H of saturated solution	8.2	7.9	8.5	8.2
Crystal form	Orthorhombic	Monoclinic	Radiated columnar, monoclinic	Monoclinic (twinned crystals and clusters)

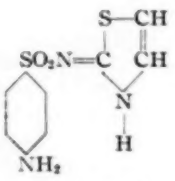
Table I shows the analytical data on *dl*-desoxyephedronium sulfathiazole, together with three other similar compounds. Under ordinary conditions, the crystals are stable, but on heating the compound readily decomposes to form desoxyephedrine alkaloid and sulfathiazole. As the alkaloid is volatile at elevated temperatures, caution must be observed in handling and drying the compound to avoid loss. Considerable difficulty was encountered in making sulfur analyses on desoxyephedronium sulfadiazine because of the fact that the crystalline material precipitated from aqueous solution contains approximately 7.5% water. Sulfur results obtained by fusion with sodium carbonate and sodium nitrate, and precipitation of the resultant sulfate with barium chloride, will be low unless the material is thoroughly dried. However, if the material has been heated during the drying process, sulfur determinations will be high due to loss of desoxyephedrine from the compound. Best results were obtained by simply allowing the compound to remain in a desiccator, at room temperature, for several days.

Similarly, some difficulty may be encountered in determining the melting point of the product and the sealed capillary tube method is recommended in order to prevent excessive loss of desoxyephedrine vapor. The hot-plate type melting-point apparatus is not satisfactory because the compound progressively decomposes (without charring) until only sulfadiazine remains.

The substituted sulfonamides probably exist in several tautomeric forms, as may be indicated for sulfathiazole:



Structure *A*



Structure *B*

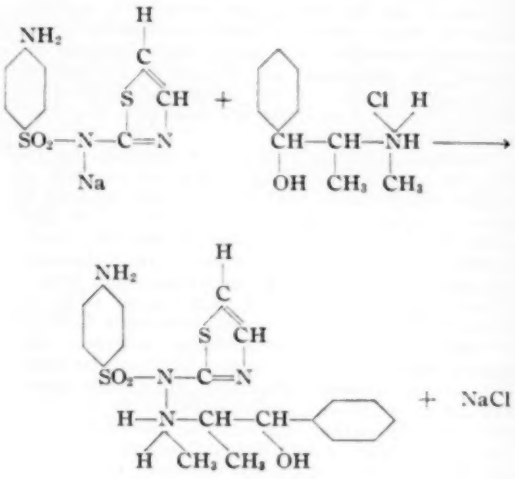
In all probability, Structure *A* represents the form encountered in the new compounds discussed in this paper because:

1. The substituted sulfonamides are amphoteric, and Structure *B* should only form salts with acids as it is in reality a diamine.
2. Dissociation of the onium salts gives the aqueous solutions *p*H's which are in the same range though slightly lower than the alkali metal salts of the substituted sulfonamides, indicating linkage to the amide nitrogen.
3. Acidification of the aqueous solution of the onium salts causes precipitation of the substituted sulfonamides at the same approximate *p*H as occurs with the alkali metal salts of the sulfonamides.
4. Vasosulfa compound (9) synthesis by double decomposition, discussed later in this paper, further indicates that tautomeric form *A* is the reactant in onium salt formation because the sodium is replaced by the pressor amine.

Compound formation between sulfonamides and vaso-constrictive amines appears to be quite general. Sulfanilamide appears to enter into an addition reaction with vaso-constrictors, but because its acidic properties are far less pronounced than those of the substituted sulfonamides such as sulfadiazine, sulfathiazole and sulfapyridine, succinyl sulfathiazole, etc. (10), its -onium salts are less stable and more difficult to isolate. To date we have not succeeded in making addition compounds in aqueous solutions with sulfaguanidine because of the relatively very low solubility of this drug in alkaline solutions.

The procedure for making ephedronium sulfathiazole, ephedronium sulfadiazine, and desoxyephedronium sulfadiazine is identical with that described above for the preparation of desoxyephedronium sulfathiazole. Because of the fact that the sulfonamide is relatively acidic in aqueous solution, and the vaso-constrictor amine is relatively basic in aqueous solution, -onium salt formation is similar to the general chemical reaction between ammonia and an acid to form a salt.

Vasosulfa compounds may also be readily and conveniently synthesized by a double decomposition reaction in aqueous solution involving the sodium salt of the sulfonamide and an acid salt of the vaso-constrictor. Thus, sodium sulfathiazole reacts in aqueous solution with ephedrine hydrochloride according to the following equation:



Present indications are that the vasosulfa compounds may be a very useful, physiologically active, group of compounds. The toxicity of desoxyephedronium sulfathiazole has been measured by Richards and has been found to be relatively low. A 1% aqueous solution of *dl*-desoxyephedronium sulfathiazole was injected intravenously in mice with the following results:

Death occurred in 1 mouse out of 10 on 87 mg./Kg.; in 4 mice out of 10 on 98 mg./Kg.; in 7 mice out of 17 on 109 mg./Kg.; in 3 mice out of 3 on 150 mg./Kg.

The limited number of animals used does not permit definite conclusions, but from the present data the impression is gained that the desoxyephedronium sulfathiazole compound is certainly not more toxic than an equal amount of desoxyephedrine alone (11). Toxicity measurements on the other compounds reported in this paper will be reported at a later date.

The high physiological activity of desoxyephedronium sulfathiazole is beneficial because only a very small amount is necessary to produce the desired clinical result. Undesirable side effects such as sleeplessness, nervousness, tachycardia, sneezing and the like are practically unknown when only 1/4% *dl*-desoxyephedrine hydrochloride is used in a solution of sodium sulfathiazole for nasal drops or spray.

The formation of desoxyephedronium sulfathiazole

makes possible the formulation of a very useful solution for intranasal therapy:

Sodium sulfathiazole sesquihydrate	2.5%	by weight
Sodium sulfite anhydrous C.P.	2.0%	by weight
Glycerin	1.0%	by volume
dl-Desoxyephedrine hydrochloride	0.125%	by weight

Heat the above ingredients with sufficient water to dissolve, and add 0.8% 0.6 *N* hydrochloric acid by volume, or sufficient acid to bring the *p*H of the solution to approximately 8.7. Sterilize by boiling, and add flavoring and coloring ingredients to suit.

After cooling, the *p*H of the finished stabilized solution is approximately 9.0. Prolonged exposure to sunlight tends to increase the *p*H of the solution slightly. Similarly, prolonged exposure to air tends to decrease the *p*H of the solution somewhat. Since these two factors work in opposite directions, only very unusual treatment will cause the *p*H of the solution to vary above or below the indicated value.

In packaging the solution for commercial use, the selection of high-grade glass bottles is essential. Unless chemically resistant glass is used, precipitation will occur in the solution as a result of long storage. The crystals formed are apparently silica sulfathiazole. Analytical work has not as yet been completed on this product, but is planned for presentation at a later date. The use of cork or rubber stoppers should be avoided, as contact of the stabilized solution with either of these materials appears to cause serious discoloration. Preferably, the bottles should be sealed with plastic caps equipped with an inert inner liner such as wax or suitable resins.

The solutions formulated above have been subjected to many clinical trials with favorable results. A separate publication (12) will show that the solution has been found to give definite relief for infections of the upper respiratory tract, as has been proved by observations in over 1000 individual cases. A modified formulation, using only 1.0% sodium sulfathiazole with 0.75% sodium sulfite and 0.1% desoxyephedrine hydrochloride has been found beneficial for ophthalmic use, and has given definite relief in many types of eye infections (13).

SUMMARY

1. Sodium sulfite, together with glycerin, is effective as a stabilizer for aqueous sodium sulfathiazole solutions.
2. Four new sulfonamide compounds are reported.
3. The formulation of a new therapeutic solution for intranasal therapy is presented and discussed.

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Studies on Indian Rhubarb. I*

By Heber W. Youngken

Upon learning in the fall of 1942 that a shortage of Chinese Rhubarb threatened the country owing to the cessation of importations, the Subcommittee on Botany and Pharmacognosy of the U. S. Pharmacopœia Revision Committee, after investigation, ascertained that the stock pile of available U. S. P. Rhubarb was dangerously short. While this committee began to look about for a substitute which might be recommended for admission for the duration of the emergency, an Indian Rhubarb was offered for entry into this country but was held up by the government because it did not conform to the U. S. P. definition for Rhubarb.

Lots totaling about 20 pounds were submitted by the Food and Drug Administration and two crude drug importing houses for comparison with U. S. P. XII Rhubarb.

A preliminary examination of these lots showed them to be of similar general character. Each of them consisted of segments of unpeeled roots of dark brown to purplish brown color externally, with a radiate wood, no visible stellate spots, and devoid of the characteristic odor of Chinese Rhubarb and with a more astringent taste. Each lot, when examined by the Hanovia Lamp under filtered ultraviolet light, exhibited a velvety brown fluorescence similar to Chinese Rhubarb (F.g. 1). The powdered drug met the

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Presented to the Scientific Section of the A. PH. A., Columbus meeting, 1943.

tests for anthraquinone compounds and for emodin and chrysophanic acid of the U. S. P. It also met the U. S. P. standard for diluted alcohol-soluble extractive (1).

Sources of Indian Rhubarbs.—According to Kirtikar and Basu (2) the species of *Rheum* occurring in the Himalaya Mountains of India are *Rheum spiciforme* Royle, *R. moorcroftianum* Royle, R. *emodi* Wallick, R. *acuminatum* Hooker filius & Thom and *R. webbianum* Royle. It is likely that *Rheum acuminatum* is merely a form of *Rheum Emodi*, for these authors state that "according to J. D. Hooker (3) "it is probably only a small form of *Rheum emodi* Wall. *croftianum* and *R. webbianum*. Dymock (5) in "Pharmacographia Indica" writes "In the Pharmacopœia of India the bazaar rhubarb of India is attributed to *Rheum emodi*, *R. moorcroftianum* and *R. webbianum*, all Himalayan species." In 1928 Dutt (6) stated:

> The ordinary rhubarb of the drug shops is mostly a Chinese product, but some quantity of Indian Rhubarb also finds its way to the market and the quantity is increasing since the time of the war. It is said that the small, odorless transverse segments of the drug found in the country drug shops are derived from *Rheum webbianum*, while the large, twisted or cylindrical and fairly odorous ones belong to *Rheum emodi*. It would appear, however, that genuine Emodi segments are scarce among the collections made by the hill people in the higher ranges of the Himalayas. The Himalayan drug differs from the Chinese in being not decorticated, darker in color, coarser in texture, and in giving a brownish yellow powder instead of the bright yellow powder of the Chinese Rhubarb.

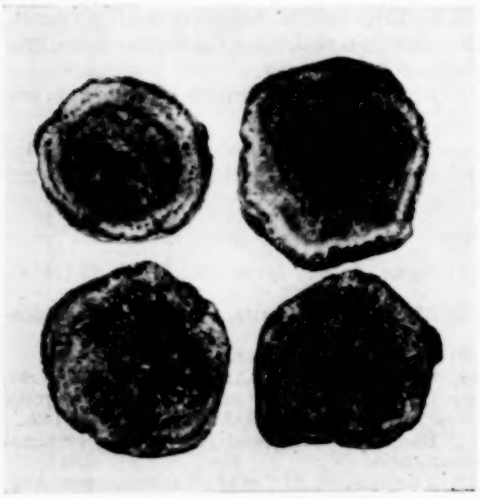

Fig. 1.—Chinese Rhubarb.

A, smoothed transverse surfaces of rhizomes of *Rheum palmatum* type occurring in U. S. P. Rhubarb. *B*, smoothed transverse surfaces of rhizomes of *Rheum officinale* type, occurring in commercial U. S. P. Rhubarb.

Fig. 2.—Indian Rhubarb. Segments of Roots of Commercial Drug.

with acuminate leaves, but flowers considerably larger." The roots of these species are stated to constitute the principle portion of Indian or Himalayan Rhubarb (Fig. 2). They describe two principal varieties of Indian or Himalayan Rhubarb, as follows:

> The large (from *R. Emodi?*) occurs in twisted or cylindrical pieces of various sizes and shapes, furrowed, cut obliquely at the extremities, about 4 in. long and $1^1/_2$ in. in diameter, of a dark brown color, feeble rhubarb odor and bitter astringent taste; texture radiated, rather spongy, not presenting on fracture the marbled texture of ordinary rhubarb; pulverized with difficulty; powder of dull brownish yellow color. The small (from *Rheum Webbianum*) consists of short transverse segments of the root branches; of a dark brownish color, odourless or nearly so with a very bitter, astringent taste. Both kinds are liable to considerable variations in physical characters.

Watt (4) states the chief sources of Himaayan rhubarb are *Rheum emodi*, *R. moor-*

While several attempts to obtain botanically authenticated rhizomes of the various Himalayan rhubarbs from reliable sources in India have been unproductive up to this time owing to war conditions, the author did obtain a dried museum sample from Dr. Graf of the Smithsonian Institution. This sample was selected by Dr. Charles Whitebread from material available in the materia medica collection, and he furnished the following statement regarding it: "No. 141924. *Rheum moorcroftianum* Royle, from Himalaya Mountains. This specimen was in the Indian Exhibit at the Paris Exposition of 1878. From the Museum of the East India

Company, London." This specimen represented a short segment of the root having a dark brown external color, a radiate, brown wood, and a very bitter astringent taste. It was devoid of odor. It differed microscopically from the Indian Rhubarb roots which were submitted to the author as representative of recent importations of Indian Rhubarb.

Until botanically authenticated Indian Rhubarb roots of known origin can be obtained for comparison with the commercial material being received in this country from India, no botanical determination of specific source or sources of the latter appears possible. From the meager descriptions of the roots of Himalayan species of *Rheum* in the literature, the present commercial material resembles those of *Rheum webbianum* more than the others. Seedlings of *Rheum webbianum* have been germinated from seeds of that species by the writer and young plants potted and later planted in the Medicinal Plant Garden of the Massachusetts College of Pharmacy. Freshly dug root systems of the following Asiatic species which were grown from seed sent to Dr. M. F. Babb of the Cheyenne, Wyo., Horticultural Field Station, by the F. P. I. office have recently been received: *Rheum franzenbachii* Muent., *R. webbianum* Royle, *R. undulatum* L. and *R. emodi* Wall. Some of this material has been planted in the college garden and the remainder preserved for future study. Since rhubarb does not always come true to type from seed, these roots could not be guaranteed to be true to the species name, thus making it necessary to defer verification of their identity until the plants come into flower. Studies are contemplated on the rhizomes and roots of these in comparison with those of commercial Indian Rhubarb as soon as the plants have sufficiently matured and are studied as to actual identity.

Description of Commercial Indian Rhubarb.—Unground Indian Rhubarb occurs in unpeeled or partially peeled, subcylindrical or irregular pieces, mostly of roots, which range from 2 to 20 cm. in length and from 1.5 to 8 cm. in diameter. Its outer surface varies from dark brown to purplish brown or from yellowish brown to reddish brown where the cork is abraded. It is irregularly longitudinally wrinkled, furrowed or ridged, some pieces also showing transverse wrinkles or annulations. Most of the pieces are moderately firm in texture. The fracture is uneven and granular, the fractured surface having a dull orange to yellowish brown color. The smoothed transverse surface shows a dark brown cambium line separating a narrow bark from a broad, radiate wood, the latter frequently exhibiting annual rings. The odor is faintly aromatic, the taste bitter and more astringent than Chinese Rhubarb.

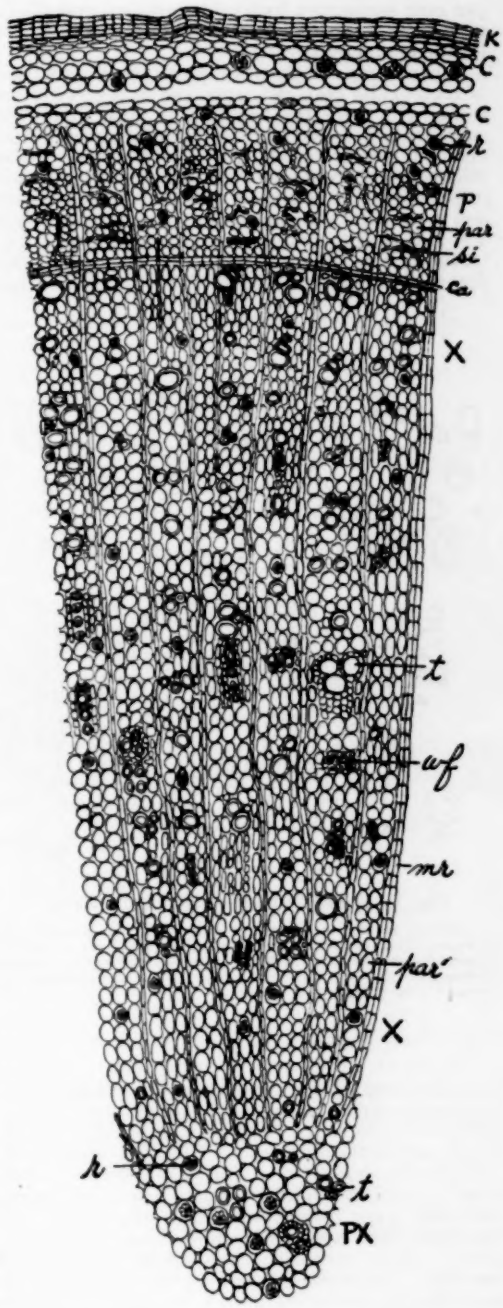

Fig. 3.—Indian Rhubarb Root. Representative Portion of a Transverse Section of Typical Commercial Drug.

K, cork; *C*, cortex; *P*, phloem; *Ca*, cambium; *X*, xylem (secondary); *PX*, primary xylem; *r*, rosette aggregate of calcium oxalate; *si*, sieve tubes; *par*, phloem parenchyma; *par'*, wood parenchyma; *t*, tracheae; *mr*, medullary ray.

Histology of Commercial Indian Rhubarb Root.—Transverse (Fig. 3) and radial-longitudinal sections, separately mounted in water, phloroglucin-HCl, in chloral hydrate solution, cyanin solution, and in 5 per cent potassium hydroxide solution and studied under the compound microscope, presented the following characteristics:

A bark zone composed of (*a*) an outer cork region of rectangular cork cells arranged in regular radiate rows, with suberized walls and dark amorphous contents, (*b*) a secondary cortex of a variable number of layers of cortical parenchyma, many of the cells of which contain rosette aggregate crystals of calcium oxalate or starch grains, a number tannin and a resinous substance, and (*c*) a broad phloem. The last region comprises most of the bark. It is traversed by numerous radiate and irregularly slightly undulate to straight phloem rays which separate phloem strands, the cells of which contain rosette aggregate crystals of calcium oxalate, starch, tannin and resinous material. A prominent wavy cambium line separates the narrow bark from the broad wood. The wood is divided into a large number of narrow wood wedges by slightly undulate, radiating xylem medullary rays from 1 to 3 cells in width. The medullary rays contained yellow masses of anthraquinone compounds which were colored red by KOH solution. The wood wedges are made up of wood parenchyma containing starch, tannin and rosette aggregates of calcium oxalate, the latter up to 132 μ in diameter, and tracheae of reticulate and pitted character with nonlignified walls. The reticulate tracheae were up to 133 μ in diameter, and constituted the main form of vessel. The tracheae occur in radiate and circular to oval groups and isolated.

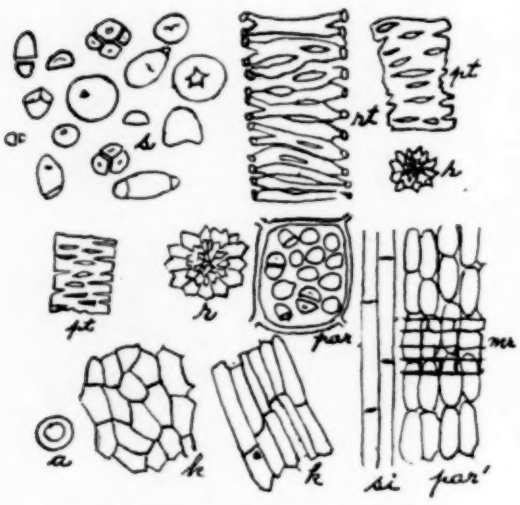

Fig. 4.—Powdered Indian Rhubarb Root.

s, starch grains; *a*, altered starch grain; *vt*, fragment of reticulate trachea; *pt*, fragment of pitted trachea; *r*, rosette aggregate of calcium oxalate; *par*, starch-bearing parenchyma cell; *h*, cork; *si*, sieve tubes; *par'*, parenchyma traversed by medullary ray (*mr*).

Powdered Indian Rhubarb.—The powdered Indian Rhubarb was of a dull, moderate to dark yellowish orange color in contrast to the bright yellowish orange to yellowish brown color of Chinese Rhubarb. It showed, upon microscopical examination (Fig. 4); numerous rosette aggregate crystals of calcium oxalate, most of which measured up to 85 μ in diameter but some were up to 132 μ in diameter. The starch grains were numerous and single to 2- to 4-compound, the individual grains spheroidal, plano-convex or angular convex, with a central to frequently excentric hilum, and up to 30 μ in diameter. The hilum varied from spheroidal, crescent-shaped, stellate to the shape of the wings of a bird in flight. Numerous nonlignified reticulate and fewer spiral tracheae and tracheids and numerous fragments of starch- and tannin-bearing parenchyma, and medullary rays were present. Many of the medullary ray cells contained an amorphous yellow to brown substance which dissolved in ammonia T. S. with a pink to red color. Many fragments of cork tissue with brownish amorphous contents were present, considerably more than in Chinese Rhubarb.

Comparison of Preparations made from U. S. P. Rhubarb and Indian Rhubarb.—In order to ascertain the suitability of Indian Rhubarb in U. S. P. and N. F. preparations of Rhubarb, the writer examined two sets of these galenicals which were prepared in the Department of Pharmacy of the Massachusetts College of Pharmacy from materials previously examined and verified by the author. The tabulation on the opposite page will indicate their contrasting organoleptic characteristics:

From the pharmaceutical standpoint, it would seem, after a study of these comparisons, that mixture of rhubarb and soda and aromatic tincture of rhubarb made from Indian Rhubarb might be less readily detected, when used in refill prescriptions, than the other preparations of Indian Rhubarb.

SUMMARY AND CONCLUSIONS

1. The results of a pharmacognostical study of commercial Indian Rhubarb recently offered for entry into the United States are reported.

2. It is shown that while this Indian Rhubarb met the chemical tests for Rhubarb as described in the U. S. P. XII, it differed from it chiefly in its unpeeled character and in its odor, color and more astringent taste.

3. The sources of various rhubarbs of Indian commerce are discussed with references to the literature and, while the botanical identity of the material examined is still undetermined, it resembles *Rheum webbianum* roots more than those of other species described in the literature.

4. A description of the unground commercial Indian Rhubarb recently received in this country is given.

5. A description of the histology of this Indian Rhubarb is given.

6. Powdered Indian Rhubarb is described and contrasted with powdered Chinese Rhubarb. Its rosette aggregate crys-

tals of calcium oxalate were found to be up to 132 μ in diameter whereas those of Chinese Rhubarb are up to 190 μ. Its individual starch grains attain a greater size than those of U. S. P. Chinese Rhubarb. Considerably more fragments of cork tissue occur in the Indian than the Chinese variety.

7. A comparison is made of the color, odor and taste of galenical preparations of Rhubarb official in the U. S. P. and N. F. which were prepared from Chinese and Indian rhubarbs. It is shown that these differ from each other in one or more organoleptic features, the greatest differences being found in the Compound Powder of Rhubarb made from each variety.

U. S. P. RHUBARB	INDIAN RHUBARB
Fluidextract of Rhubarb	Fluidextract of Rhubarb
Color: Reddish brown	Color: Reddish brown
Odor: Slight, characteristic	Odor: Alcoholic
Taste: Bitter, astringent, with flavor of rhubarb	Taste: Bitter, more astringent than that made from U. S. P. rhubarb, and devoid of the rhubarb flavor
Tincture of Rhubarb	Tincture of Rhubarb
Color: Brownish red	Color: Reddish brown
Odor: Aromatic, characteristic	Odor: Alcoholic
Taste: Bitter, astringent and with a rhubarb flavor	Taste: Astringent, bitter and devoid of rhubarb flavor
Compound Powder of Rhubarb	Compound Powder of Rhubarb
Color: Pale pink	Color: Deep pink
Odor: Slightly fragrant	Odor: Nil
Taste: Characteristic	Taste: Similar
Syrup of Rhubarb	Syrup of Rhubarb
Color: Red	Color: Deep red
Odor: Aromatic, characteristic	Odor: Distinctly different from official syrup
Taste: Aromatic, cinnamon-like and very slightly astringent	Taste: Aromatic, cinnamon-like and moderately astringent
Mixture of Rhubarb and Soda	Mixture of Rhubarb and Soda
Color: Light reddish brown	Color: Brownish red
Odor: Peppermint-like	Odor: Peppermint-like
Taste: Aromatic, characteristic	Taste: Similar
Aromatic Tincture of Rhubarb	Aromatic Tincture of Rhubarb
Color: Dark reddish brown	Color: Dark brown
Odor: Characteristically aromatic	Odor: Similar
Taste: Aromatic, astringent and bitter	Taste: Slightly aromatic, very astringent and intensely bitter
Aromatic Syrup of Rhubarb	Aromatic Syrup of Rhubarb
Color: Light brownish red	Color: Reddish brown
Odor: Aromatic	Odor: Aromatic, similar
Taste: Sweet, aromatic and very slightly astringent	Taste: Sweet, aromatic and very astringent

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A Pharmaceutical Study of the Purity and Preservation of American Oils of Turpentine*,†

By W. T. Sumerford, with the technical assistance of A. B. Huff and Otha K. Coleman

It is recognized that the therapeutic value of oil of turpentine is limited (1). However, a monograph on the oil and another on the rectified oil are included in the twelfth revision of the U. S. Pharmacopœia (2), and several preparations of these oils are officially recognized in this country. Oil of turpentine, which is obtained from both the oleoresin (gum) and wood of several species of the pine, is the chief item in the Naval Stores industry of the South, and as a result of its abundance the oil is widely used as an internal and an external medicament by the laity. It is, moreover, one of the principal ingredients in several proprietary medicinal preparations.

This study of oil of turpentine had four objectives: (*a*) to determine which of the six common, commercial, American oils of turpentine, before and after rectification, conform to the requirements of the U. S. Pharmacopœia for Oil of Turpentine and Rectified Oil of Turpentine, respectively; (*b*) to determine the relative resistance of these oils to physical and chemical changes, and the best conditions for storing them to retard these changes; (*c*) to discover an efficient and practical preservative for these oils; and (*d*) to survey critically the U. S. Pharmacopœia monographs on Oil of Turpentine and Rectified Oil of Turpentine.

The specifications of the U. S. Pharmacopœia XII for Oil of Turpentine and Rectified Oil of Turpentine, which are virtually identical with those of the U. S. Pharmacopœia XI (3), are stated briefly in Table I.

PART I. PURITY OF SOME AMERICAN OILS OF TURPENTINE WITH RESPECT TO THE TESTS OF THE U. S. PHARMACOPŒIA XII

Most of the previous work done to determine the composition, purity and properties of the various oils of turpentine produced in different countries is from a commercial rather than a medicinal standpoint (4). A more recent report from Germany deals with five German and five foreign oils (5).

EXPERIMENTAL

Fresh samples of six commercial American oils of turpentine, before and after rectification, were subjected to the tests outlined in Table I. The results of these tests are given after the description of each oil and rectified oil, respectively.

Oil No. 1. Steam Distilled Wood Turpentine Oil.—This oil is obtained chiefly by extracting shredded stumps of the longleaf yellow pine (*Pinus palustris* Miller) and/or the Cuban or slash pine (*P. caribaea* or *heterophylla* Morelet) with a petroleum solvent at a maximum temperature of 120° C. The crude terpene oils are separated from the solvent, chemically treated and then fractionated to give the commercial product which is known as "steam and solvent turpentine."

(It should be pointed out here that the U. S. Pharmacopœia definition, Specification *A* in Table I, denies official recognition to all oils of turpentine obtained from wood.)

With the above exception, which is to be understood throughout this paper, this oil met all requirements of the U. S. Pharmacopœia XII except in specific gravity (0.852) and refractive index (1.4672). While the odor of this oil is characteristic of turpentine, it is possible to distinguish its odor from that of an oil obtained from the oleoresin. The American Society for Testing Materials resolves this difficulty by stating in their specifications that the odor of a sample of turpentine oil shall be characteristic of the type specified, *i. e.*, wood or oleoresin.

A quantity of this oil and of each of the other five oils was rectified by the U. S. Pharmacopœia XI method which consists of treating some of the oil with an equal volume of 5% sodium hydroxide solution, distilling off three-fourths of the oil, separating it from water, and finally drying it over anhydrous sodium sulfate.

After rectification the sample of oil No. 1 conformed to all requirements of the U. S. Pharmacopœia XII for Rectified Oil of Turpentine except in refractive index (1.4652).

Oil No. 2. Destructively Distilled Wood Turpentine Oil.—This oil is one of the first fractions obtained by distilling the resinous wood of several species of *Pinus*. The oil is further purified, but is generally considered unfit for medicinal purposes.

This oil failed to conform to 4 of the 13 official tests. Its specific gravity was 0.852; only 88% distilled between 154–70° C.; and it failed with respect to both tests for other foreign substances.

After rectification this oil failed to meet 3 of the 13 U. S. Pharmacopœia XII requirements for Rectified Oil of Turpentine. Its specific gravity was 0.847; it contained 0.023 Gm. of residue per 5 cc. of oil; and it contained foreign substances B.

Oil No. 3. Fire-Distilled Dehydrated Gum Turpentine Oil.—This oil is obtained from the oleoresin of the living trees, *P. palustris* and/or *P. caribaea*, by distilling it with a little water in a large copper kettle heated directly with fire. Afterward the oil is separated from the water and dried with sodium chloride (6).

* Received Aug. 28, 1943, from the School of Pharmacy, University of Georgia, Athens, Ga.

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Thanks are due Dr. G. P. Shingler of the Naval Stores Research Division of the U. S. D. A., Nelio Resin Processing Co., Southern Pine Chemical Co., and Newport Industries, Inc., for the samples of oil; and The American Can Co., Aluminum Company of America, and Owens-Illinois Glass Co. for the containers which were used in this study.

This oil met all U. S. Pharmacopœia XII requirements for Oil of Turpentine, and after rectification it met all requirements for Rectified Oil of Turpentine.

Oil No. 4. Fire-Distilled Undehydrated Gum Turpentine Oil.—This oil is identical with oil No. 3 except that it is not dehydrated with sodium chloride. This is the type oil produced by operators in the small individual distilling outfits.

This oil met all U. S. Pharmacopœia XII requirements for Oil of Turpentine, and after rectification it met all requirements for Rectified Oil of Turpentine.

Oil No. 5. Steam-Distilled Dehydrated Gum Turpentine Oil.—This oil is produced by distilling the oleoresin of *P. palustris* and/or *caribaea* in an experimental still heated with steam (7). The oil is then dehydrated with sodium chloride.

This oil met all U. S. Pharmacopœia XII requirements for Oil of Turpentine, and after rectification it met all requirements for Rectified Oil of Turpentine.

Oil No. 6. Vacuum-Distilled Gum Turpentine Oil.—This oil is produced in large central distilling plants by distilling precleaned oleoresin of *P. palustris* and/or *caribaea* under reduced pressure with the use of steam. The number of these central plants is increasing.

This oil met all U. S. Pharmacopœia XII requirements for Oil of Turpentine, and after rectification it met all requirements for Rectified Oil of Turpentine.

It is noteworthy that the samples of oils Nos. 3, 4 and 5 were pure enough even before they were rectified to meet all U. S. Pharmacopœia XII requirements for Rectified Oil of Turpentine.

PART II. THE RELATIVE RESISTANCE OF SOME AMERICAN OILS OF TURPENTINE TO PHYSICAL AND CHEMICAL CHANGES UNDER SEVERAL DIFFERENT STORAGE CONDITIONS

Due to the presence of unsaturated linkages and the strained ring structures in its several bicyclic hydrocarbons, oil of turpentine easily ozonizes, resinifies, polymerizes and undergoes other chemical reactions on standing which changes its composition and its physical and chemical properties and renders it unfit for oral use (8).

Using the following U. S. Pharmacopœia XII specifications and tests, each of which is given a number corresponding to its position in Table I—*1*, color; *2*, odor and taste; *3*, solubility; *4*, specific gravity; *7*, reaction; *8*, distillation range; *9*, fixed oils; *11*, mineral or rosin oils; *12*, foreign substances A; and *13*, foreign substances B—an investigation was made to determine the resistance of the six oils to the physical and chemical changes as indicated by the above tests. Although fixed oils and mineral or rosin oils were known to be absent, tests Nos. *9* and *11* were included because both tests afford an excellent means of determining the presence of a nonvolatile residue, probably a polymerization product, which develops in the oil as it ages.

TABLE I

	Specification	Oil of Turpentine	Rectified Oil
A.	Definition	The volatile oil distilled from the oleoresin obtained from *P. palustris* Miller and other species of *Pinus* (Fam. *Pinaceae*) which yield exclusively terpene oils	The same
1.	Color	Colorless liquid	The same
2.	Odor, Taste	Characteristic, becoming stronger on exposure	The same
3.	Solubility	Oil is soluble in 5 vol. alcohol	The same
4.	Specific gravity	0.854 to 0.868 at 25° C.	0.853 to 0.862 at 25° C.
5.	Optical rotation	Active, but variable	The same
6.	Refractive index	1.4680 to 1.4780 at 20° C.	The same
7.	Reaction	Alcoholic solution (1–5) neutral or only slightly acid to litmus	The same
8.	Distillation range	90% of 100 cc. distils between 154 and 170° C.	The same
9.	Fixed oils	3 drops on unsized paper leaves no stain	The same
10.	Mineral oil	Does not exceed 1% as determined by polymerization with sulfuric acid	The same
11.	Mineral or rosin oils	5 cc. contains 0.1 Gm. or less of residue after evaporation on steam bath	0.015 Gm. or less
12.	Foreign substances A	5 cc. of the oil shaken with 5 cc. KOH (5%) does not become darker than light straw yellow within 24 hrs.	The same
13.	Foreign substances B	5 cc. oil shaken with 5 cc. HCl does not become darker than light straw yellow in either layer within 5 mins.	The same

EXPERIMENTAL

A sample of each of the six oils, before and after rectification, was stored for a period of one year under these four storage conditions: (*a*) a partly filled, tightly stoppered prescription bottle; (*b*) a partly filled prescription bottle closed with a loose cotton plug; (*c*) a partly filled, tightly stoppered amber bottle; and (*d*) a tin can closed with a screw cap. All samples were stored at room temperature for 12 months, and then subjected to the above ten tests.

The results with the rectified oils are given in Table II. The results with the unrectified samples

are omitted since there were no significant differences in the behavior of the two qualities of oils under these storage conditions except possibly that the unrectified samples of the two wood oils proved to be more stable in the cotton-plugged bottles than did the corresponding rectified samples.

In a second restricted series of storage experiments it was found that samples of oil No. 6 stored in aluminum cans (9) for six months both in the presence and absence of air deteriorated rapidly according to tests Nos. *4*, *8*, *9*, *11* and *13*. An identical sample tightly stoppered and stored in a refrigerator changed, but to a lesser extent.

To observe the effects of light on oil of turpentine preserved in the absence of air, rectified samples of oils Nos. 1 and 6, selected as representatives of wood and gum turpentine oils, respectively, were put up under a vacuum in clear glass. One sample of each oil was stored in the dark and another in an east window for six months. The two samples stored in the dark showed no change according to the ten selected tests. The sample of oil No. 1 stored in the light failed to pass test No. *1* while the corresponding sample of oil No. 6 failed to pass tests Nos. *1* and *11*.

A freshly rectified sample of oil No. 6 which passed all U. S. Pharmacopœia XII tests for purity, having an exact specific gravity of 0.8576 and a residue content of 0.0089 Gm. per 5 cc., was stored in a prescription bottle under a carbon dioxide atmosphere for one year. An examination of the oil at the end of the period showed that it was practically unchanged, having a specific gravity of 0.8610 and a residue content of 0.0147 Gm. per 5 cc., and passing all other U. S. Pharmacopœia XII tests.

TABLE II.—RECTIFIED OILS OF TURPENTINE AFTER ONE YEAR OF STORAGE

Rectified Oil	Closed Prescription Bottle	Cotton-Plugged Prescription Bottle[a]	Closed Amber Bottle[a]	Closed Tin Can[a]
Steam distilled wood	None (passed all tests)	(*1*) (*4*) (*7*) (*8*) (*9*) (*11*) (*12*) (*13*)	None	None
Destructively distilled wood	None	(*1*) (*8*) (*9*) (*12*)	(*1*) (*11*) (*13*)	(*1*) (*13*)
Fire distilled gum (dehydrated)	None	(*1*) (*4*) (*7*) (*8*) (*9*) (*11*) (*12*) (*13*)	None	None
Fire distilled gum (undehydrated)	None	(*1*) (*4*) (*8*) (*9*) (*11*) (*12*) (*13*)	None	None
Steam distilled gum (dehydrated)	None	(*1*) (*4*) (*8*) (*9*) (*11*) (*12*) (*13*)	None	None
Vacuum distilled gum	None	(*1*) (*4*) (*8*) (*9*) (*11*) (*12*) (*13*)	None	None

[a] Numerals refer to the numbered specifications and tests given in Table I with which the respective oils failed to comply after one year of storage under the conditions specified.

PART III. THE PRESERVATION OF TURPENTINE OILS WITH CHEMICAL PRESERVATIVES

Since most of the changes in oil of turpentine are caused by oxidation, and in view of the fact that these reactions are influenced by moisture (10) the preservatives usually found to be effective in the oil have been antioxidants, reducing and dehydrating agents. According to previous reports the most promising of these agents are: hydroquinone (11, 12, 13), pyrogallol (12, 14), lower aliphatic alcohols (15), sodium sulfite (16), calcium oxide (16) and metallic magnesium and tin (9). The more recent reports on this subject have come from the patent literature on synthetic antioxidants developed as preservative for rubber (17, 18).

EXPERIMENTAL

The problem of finding a suitable preservative agent was approached by preparing seven samples of each of the six rectified oils in half-filled, screw-capped prescription bottles. One of the seven samples of each oil was left untreated to serve as a control, and to each of the other six samples of each oil were added, respectively, 0.1% (in the case of ethyl alcohol 0.5%) of the following preservative agents: (*a*) an aliphatic alcohol (ethyl alcohol), (*b*) a phenol (thymol), (*c*) an ether containing a phenolic hydroxyl (guaiacol), (*d*) an alicyclic alcohol (α-terpineol), (*e*) an ester (methyl salicylate), and (*f*) a cyclic ketone (camphor). The low concentration of 0.1% of the preservatives was used since appreciable amounts of these compounds could not be tolerated in medicinal turpentine oil. The prepared samples and the controls were stored for 12 months, and then unstoppered and exposed to the air for an additional period of three months (July 29 to October 20, 1942). At the end of this period all samples were tested by the ten selected official tests.

The results of these tests are shown in Table III. The numerical values are given in the case of specific gravity, distillation range, and mineral or rosin oil residue, and a capital X is placed after the value if it is not within in the U. S. Pharmacopœial XII range. In the case of the other tests a plus sign is used to indicate passing, a capital X indicates failure to pass, and a question mark indicates a border line result.

In view of the definite superiority of the phenols, guaiacol and thymol, in preserving all of the oils, rectified samples of each of these six oils were treated with 0.1% of an additional series of substituted phenols, including hydroquinone and pyrogallol which were used for comparison purposes. Several nitrogen derivatives, analogs of which are known to act as antioxidants, were included in this series of experiments. The samples, with controls, were stored in partly filled, cotton-plugged, prescription bottles at room temperature from December 11, 1942, until June 14, 1943. After this period the samples were tested by subjecting them to the simple tests Nos. *1*, *9*, *12* and *13*, and if the preserved sample looked promising with these tests it was carried through the entire series of ten tests. The most effective preservatives of those tried are listed in Table IV which gives the results of these preservatives when used with oil No. 6 (vacuum-distilled gum turpentine).

While the preservatives listed in Table IV were the most effective they represent only about one-fourth of the compounds studied. To focus more attention on these preservatives, some of the compounds which significantly failed to preserve the oil are classified and listed below.

Alcohols: glycerin, "Cellosolve" solvent, tocopherol.

Nitrogen derivatives: *N*-acetyl-*p*-aminothymo and dibenzoyl-*p*-aminothymol.

PART IV. A CRITICAL SURVEY OF THE U. S. PHARMACOPŒIA XII MONOGRAPHS ON OIL OF TURPENTINE AND RECTIFIED OIL OF TURPENTINE

Definition.—While the official tests would indicate that oil of turpentine from destructively distilled wood is inferior to that from the oleoresin, the original purity and keeping qualities of the oil obtained by extracting wood closely approach those of the oil from the oleoresin. Whether the presence of some 18% of monocyclic hydrocarbons and

TABLE III

	Color (1)	Odor and Taste (2)	Solubility (3)	Specific Gravity (4)		Reaction (7)	Distillation Range (8), % between 154–170° C.		Fixed Oils (9)	Mineral or Rosin Oils (11)		Foreign Substance A (12)	Foreign Substance B (13)
Oil No. 1. Control	?	+	+	0.982	X	X	56	X	X	2.700	X	X	X
Ethyl alcohol	+	+	+	0.933	X	+	80	X	X	1.110	X	?	X
Thymol	X	+	+	0.857		+	96		+	0.024	X	+	+
Guaiacol	+	+	+	0.857		+	96		+	0.031	X	+	+
α-Terpineol	+	+	+	0.936	X	+	70	X	X	1.310	X	X	X
Methyl salicylate	+	+	+	0.935	X	+	76	X	X	1.306	X	X	X
Camphor	+	+	+	0.938	X	+	74	X	X	1.670	X	X	X
Oil No. 2. Control	X	+	+	0.981	X	X	58	X	X	2.251	X	X	X
Ethyl alcohol	X	+	+	0.941	X	+	66	X	X	1.571	X	X	X
Thymol	X	+	+	0.929	X	+	70	X	X	1.394	X	?	X
Guaiacol	X	+	+	0.928	X	+	70	X	X	1.374	X	X	X
α-Terpineol	X	+	+	0.931	X	+	68	X	X	1.409	X	X	X
Methyl salicylate	X	+	+	0.936	X	+	66	X	X	0.609	X	X	X
Camphor (not available)													
Oil No. 3. Control	X	+	+	0.991	X	X	49	X	X	2.611	X	?	X
Ethyl alcohol	?	+	+	0.936	X	+	70	X	X	1.352	X	X	X
Thymol	X	+	+	0.865	X	+	94		+	0.029	X	+	+
Guaiacol	X	+	+	0.939	X	+	66	X	X	1.809	X	?	X
α-Terpineol	?	+	+	0.934	X	+	70	X	X	1.339	X	X	X
Methyl salicylate	?	+	+	0.937	X	+	72	X	X	1.424	X	X	X
Camphor	?	+	+	0.943	X	+	69	X	X	0.643	X	X	X
Oil No. 4. Control	X	+	+	0.993	X	X	48	X	X	2.652	X	X	X
Ethyl alcohol	?	+	+	0.937	X	+	68	X	X	1.660	X	X	X
Thymol	X	+	+	0.862		+	94		+	0.022	X	+	+
Guaiacol	+	+	+	0.861		+	94		+	0.015		+	+
α-Terpineol	X	+	+	0.938	X	+	68	X	X	1.610	X	X	X
Methyl salicylate	X	+	+	0.935	X	+	71	X	X	1.478	X	X	X
Camphor	X	+	+	0.937	X	+	68	X	X	0.613	X	X	X
Oil No. 5. Control	X	+	+	0.991	X	X	44	X	X	2.666	X	X	X
Ethyl alcohol	?	+	+	0.943	X	+	64	X	X	1.507	X	X	X
Thymol	X	+	+	0.863	X	+	94		+	0.023	X	+	+
Guaiacol	+	+	+	0.861		+	95		+	0.010		+	+
α-Terpineol	?	+	+	0.943	X	+	65	X	X	1.501	X	X	X
Methyl salicylate	?	+	+	0.940	X	+	65	X	X	1.526	X	X	X
Camphor	?	+	+	0.941	X	+	66	X	X	0.575	X	X	X
Oil No. 6. Control	X	+	+	0.990	X	X	46	X	X	2.614	X	X	X
Ethyl alcohol	X	+	+	0.944	X	+	60	X	X	1.620	X	X	X
Thymol	X	+	+	0.867	X	+	93		?	0.087	X	+	X
Guaiacol	+	+	+	0.861		+	96		+	0.025	X	+	+
α-Terpineol	X	+	+	0.939	X	+	67	X	X	1.594	X	X	X
Methyl salicylate	X	+	+	0.938	X	+	70	X	X	1.554	X	X	X
Camphor	X	+	+	0.939	X	+	70	X	X	0.568	X	X	X

Phenols: phenol, *m*- and *p*-cresols, carvacrol, *o*- and *p*-nitrophenols, resorcinol monomethyl ether, *o*-hydroxybenzyl alcohol, 2-hydroxy-3-methoxybenzaldehyde, salicylic acid, salol, dithymol sulfide, phloroglucinol and phenolphthalein.

Naphthols: β-naphthol, 3-hydroxy-2-naphthoic acid.

Ethers: anisole and beechwood creosote.

2% of alcohols and aldehydes (19) in the extracted oil renders it unfit for oral use is, of course, another question.

Solubility.—This test may serve to detect certain adulterants in turpentine oils, but it is worthless in eliminating oils which have deteriorated on aging. All of the samples in this study, even those containing 40% of nonvolatile matter, easily passed this test. The test could be strengthened by requiring that oil of turpentine dissolve in an equal volume of 95% ethyl alcohol and/or in 6 volumes of 85% ethyl alcohol.

Reaction.—This test is inconclusive. For example, a series of 5-cc. samples of three different oils, which could be said to be neutral or slightly acid to litmus, required 0.02, 1.07 and 1.81 cc., respectively, of 0.0856 *N* sodium hydroxide to neutralize it to phenolphthalein when titrated in neutral ethyl alcohol. In this connection it should be pointed out that the German Pharmacopœia of 1926 required that 2.5 Gm. of Rectified Oil of Turpentine require not more than 0.3 cc. of 0.1 *N* potassium hydroxide to neutralize it to phenolphthalein.

Mineral Oil.—While this test is probably the most practical and dependable yet used to detect petroleum products in turpentine oil, it measures low percentages of kerosene only approximately, and gasoline not at all without the use of the centrifuge.

Mineral or Rosin Oils.—This test is most useful in determining the amount of nonvolatile material formed in an aged oil in addition to being a test for mineral or rosin oils. The terminal point of the evaporation is difficult to detect, however, especially when a sample contains a large amount of nonvolatile material. More reproducible results could be obtained by requiring a definite period of evaporation and desiccation to a constant weight. It was also found that saturation of the sample of oil with water prior to evaporation increased its amount of residue as much as 16% as shown by this test. This is significant in view of the fact that dehydrated and undehydrated oils are marketed.

Foreign Substances A.—To determine the results of this test by observing the color of only the oily layer leads to error since the

TABLE IV

	Color (1)	Odor and Taste (2)	Solubility (3)	Specific Gravity (4)		Reaction (7)	Distillation Range (8), % between 154–170° C.		Fixed Oils (9)	Mineral or Rosin Oils (11)		Foreign Substance A (12)	Foreign Substance B (13)
Oil No. 6. Control	X	?	+	0.974	X	X	57	X	X	2.376	X	X	X
Guaiacol	+	+	+	0.864	X	+	92		+	0.031	X	+	?
o-Cresol	X	+	+	0.893	X	X	90		+	0.371	X	X	X
Thymol	X	+	+	0.892	X	X	86	X	+	0.426	X	?	X
p-Chlorothymol	X	+	+	0.917	X	X	78	X	X	0.925	X	X	X
Hydroquinone	+	+	+	0.862		+	92		+	0.008		+	+
Pyrogallol	X	+	+	0.864	X	+	92		+	0.008		+	?
α-Naphthol	X	?	+	0.863	X	+	92		+	0.013		+	+
Resorcinol	X	+	+	0.872	X	+	92		+	0.153	X	+	X
p-Methoxyphenol	+	+	+	0.862		+	92		+	0.018	X	+	+
Catechol	X	+	+	0.862		+	92		+	0.019	X	+	+
Quinhydrone	X	+	+	0.866	X	+	95		+	0.042	X	+	+
p-Nitrosothymol (0.05%)	X	+	+	0.865	X	+	94		+	0.024	X	+	?
p-Nitrosophenol sodium salt (0.05%)	+	+	+	0.863	X	+	90		+	0.020	X	+	+
m-Aminophenol (0.05%)	X	+	+	0.863	X	+	90		+	0.018	X	+	+
p-Benzylaminophenol	X	+	+	0.862		+	92		+	0.014		+	+
Phenyl α-naphthylamine	X	+	+	0.865	X	+	92		+	0.040	X	?	?
2-Hydroxybenzylidene-4-aminothymol	X	+	+	0.867	X	+	92		?	0.042	X	+	+
4-Methoxybenzylidene-4-aminothymol	X	+	+	0.865	X	+	89	X	+	0.030	X	+	+
3-Methoxy-4-hydroxybenzylidene-4-aminothymol	X	+	+	0.865	X	+	88	X	+	0.029		+	+

emulsification occurring during the 24 hours renders this layer opaque regardless of the purity of the oil. It was our experience that this test would not be made too severe by requiring that both the alkaline and oily layers be observed for color.

Foreign Substances B.—This test, which probably detects higher-boiling polymers in aged oils, is the strictest official test for such oils. It was observed that considerable heat is produced upon the addition of the acid. The amount of heat produced and the depth of color are in a direct ratio.

Storage.—It is known that certain metals catalyze the changes which cause deterioration of turpentine oil (9, 16). Our results would indicate that glass containers are best for storing this oil, tin containers are almost as good, and aluminum containers are unsatisfactory.

SUMMARY AND CONCLUSIONS

1. Samples of two American oils of turpentine from wood and four from the oleoresin have been described and tested for purity, before and after rectification, by the U. S. Pharmacopœia XII tests.

2. Of the six oils examined the four oleoresin oils were definitely purer than the oils obtained from wood by extraction and destructive distillation, respectively, although the purity of the wood oil obtained by extraction approaches that of the oils from the oleoresin.

3. As judged by the ten official tests selected to determine purity, the wood oils and the oleoresin oils were about equal in their stability with respect to the number of changes undergone under various storage conditions, but the wood oils changed to a greater degree.

4. Light had little influence on the stability of the oils, but the amount of air in contact with the sample profoundly affected it in all cases. Closed tin containers are about equal to glass for the preservation of these oils, but aluminum containers proved unsatisfactory for this purpose.

5. Under the influence of chemical preservatives, the sample of oil obtained by the extraction of wood held up better than did the other samples, while the destructively distilled wood oil was the most difficult to preserve. The most effective chemical preservatives were in the order named: hydroquinone, *p*-benzylaminophenol, hydroquinone monomethyl ether, catechol, guaiacol and *p*-nitrosophenol sodium.

6. A survey has been made of the U. S. Pharmacopœia XII monographs on Oil of Turpentine and Rectified Oil of Turpentine, and several changes have been recommended with respect to these monographs.

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Dihydroxypropyl Bismuthate*

*By Larry M. Wheeler,** R. A. Kuever,† E. G. Gross‡, and R. Nomland§*

The value of bismuth compounds as therapeutic agents has been established. Originally, their medicinal use was confined largely to topical activity. With the recognition of the value of bismuth in the treatment of syphilis, a large number of compounds, both soluble and insoluble, have been introduced for systemic medication. This trend has brought about the development of organic preparations which are said to owe their efficacy to their bismuth content. The purpose of this report is to describe a series of soluble, organic bismuth compounds which are of value for oral administration by virtue of appreciable systemic absorption. A review of the literature reveals that desirable bismuth compounds for oral administration should be pentavalent, capable of forming neutral aqueous solutions, and be reasonably stable in the fluids of the body (1, 2, 3, 4, 5, 6).

A series of such compounds has been prepared including dihydroxypropyl bismuthate, trihydroxybutyl bismuthate, the three isomeric pentahydroxyhexyl bismuthates, and pentahydroxycyclohexyl bismuthate. Thus far only the dihydroxypropyl bismuthate has been studied pharmacologically and clinically, but studies are now in progress to determine the effects of related compounds in the treatment of rabbit syphilis. In addition, the trihydroxybutyl and the pentahydroxyhexyl bismuthates have been given orally to rats to establish the *dosis tolerata* and to demonstrate bismuth absorption from the intestines. The above compounds have all been prepared and isolated by a procedure patterned after the following description of the preparation of dihydroxypropyl bismuthate.

A review of the literature describing the desired properties lead to the selection of sodium bismuthate as the parent substance. It represents pentavalent bismuth and is energetic in responding to esterification reactions. The chemically pure variety is the most desirable, reacting completely to give pure products. The presence of hydroxyl groups is essential to render the pentavalent bismuth compounds sufficiently soluble in aqueous media. Products which have less than two hydroxyl groups in the molecule are too sparingly soluble and usually, in aqueous media, decompose too easily.

EXPERIMENTAL

Dihydroxypropyl bismuthate is prepared by the carefully controlled esterification of glycerin and sodium bismuthate.

$$C_3H_5(OH)_3 + NaBiO_3 \longrightarrow C_3H_5(OH)_2BiO_3 + NaOH$$

Sodium bismuthate is added slowly, in small portions, to the glycerin with constant stirring. The temperature is controlled between 52–55° C. Decomposition occurs at higher temperatures. The reaction is complete when all of the sodium bismuthate is dissolved, forming a clear, light tan fluid. The sodium hydroxide formed by the reaction is then neutralized with citric acid. Before neutralization, the reacted mixture has a *p*H of 11.8 showing a high degree of alkalinity. After neutralization, the *p*H is lowered to 7.2, which is that of a solution of sodium citrate.

Dihydroxypropyl bismuthate is separated from the sodium citrate by three successive steps of precipitation by means of an excess of 95% ethyl alcohol. The neutralized fluid is slowly poured, with constant stirring, into approximately five times its volume of alcohol. The precipitate is heavy and flocculent. Filtration is facilitated by use of a Büchner funnel. For the second and third precipitations, the compound is dissolved in three times its weight of water and again poured into 95% ethyl alcohol. The yield is 90–95% of the theoretical.

Dihydroxypropyl bismuthate is a light tan powder, or thin transparent amber scales depending upon the method of preparation. It has a characteristic odor and a sweet, metallic taste. Its melting point is between 240–245° C., with decomposition. Dihydroxypropyl bismuthate is very soluble in water, quite soluble in glycerin and insoluble in acetone, ethyl alcohol, ether, chloroform, petroleum ether and benzene. It is so soluble in water that all attempts to crystallize it have thus far failed. Highly concentrated aqueous solutions may readily be scaled by the usual methods. Aqueous solutions are fractionally decomposed by sunlight upon long exposure, but are stable for several months when kept in the dark. An aqueous solution has a *p*H of 6.8, being therefore slightly acidic. Solutions of dihydroxypropyl bismuthate are slowly decomposed when boiled under atmospheric pressure. Sealed ampuls, containing 3% trisodium citrate, may be sterilized without decomposition under 20 lbs. pressure, at 120° C. for 20 minutes.

COMPOSITION

When assayed for bismuth, the compound was dried to constant weight and three types of analysis were used: the U. S. P. procedure, the N. F. assay,

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** Abstract of a thesis presented to the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the degree Doctor of Philosophy, April, 1943.

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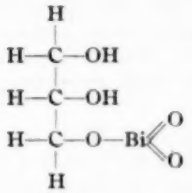
and a modification of the U. S. P. method adapted for this compound. Three samples of dihydroxypropyl bismuthate averaged 62.45% bismuth by the U. S. P. procedure, 61.76% by the N. F. assay, and 62.80% by the third procedure of direct ignition at dull redness to constant weight. Carbon and hydrogen were determined by the microanalytical procedure of Pregl (7). Using this method, three determinations averaged 11.86% carbon and 2.10% hydrogen. Since the oxygen is calculated by difference, the percentage is 23.59. These values establish the composition of dihydroxypropyl bismuthate within small experimental error. The theoretical composition and graphic formula may be illustrated thus:



Bi	62.96%
C	10.85%
H	2.12%
O	24.07%

The toxicity of dihydroxypropyl bismuthate was established orally, intramuscularly and intravenously, using rats and rabbits. Before determining the oral toxicity, the stability of the compound was studied *in vitro* using gastric juice collected from operated dogs.

Orally, dihydroxypropyl bismuthate was given by stomach tube to rats in doses corresponding to 1250 mg. bismuth per Kg., without noticeable untoward effects. The LD_{50} for rabbits, following oral administration, was established as 1000 mg. bismuth per Kg.

Intramuscularly, the rate of absorption from the site of injection is an important toxicological consideration. Injecting an aqueous solution of dihydroxypropyl bismuthate containing 3% trisodium citrate and possessing a *p*H of 7.2, no irritation or discomfort was observed. Employing an aqueous solvent, the LD_{50} is 34 mg. bismuth per Kg. Using a solvent composed of equal parts of water and propylene glycol and containing the same concentration, the LD_{50} was raised to 40 mg. bismuth per Kg. in rats.

Intravenously, the LD_{50} for rats is 8 mg. bismuth per Kg. Rabbits appear to be less resistant than rats by exhibiting an LD_{50} of 5 mg. bismuth per Kg. Dihydroxypropyl bismuthate forms stable solutions in blood serum causing neither protein precipitation nor *p*H change.

A rapid and accurate analytical procedure was adapted from Weigand's (8) modification of Leonard's (9) method of bismuth determination in combination with special methods of oxidation for each type of tissue. The colorimetric determination of the yellow to orange potassium iodobismuthate was read in a Fisher electrophotometer. The light absorption was translated into milligrams bismuth by reference curves prepared by using electrolytic bismuth metal.

The concentrated urine was oxidized with potassium permanganate and clarified with oxalic acid. Feces, organs and blood were dried and ignited in a muffle furnace at a temperature not to exceed 500° C. and the ash completely dissolved in hot sulfuric acid. Brain and spinal fluid were wet-ashed by the Newmann method employing concentrated nitric and sulfuric acids. Concentrated hydrogen peroxide was sometimes added. The carcass was ignited in a muffle furnace at a temperature not to exceed 500° C. after which the ash was totally dissolved in hydrochloric acid. The bismuth was precipitated from this solution as the sulfide and redissolved in hot sulfuric acid. This additional step of precipitation is necessary because bismuth is absorbed upon bone ash which is insoluble in the sulfuric acid. Bismuth is also absorbed upon charcoal which makes it necessary that the last trace of organic matter be completely oxidized and not removed by filtration.

Excretion studies on rats show that approximately 2% of the bismuth administered orally as dihydroxypropyl bismuthate is eliminated in the urine. Normally active university students excreted as high as 20% of the bismuth following oral ingestion: No particular consideration is necessary in connection with retention since the remainder of the bismuth which does not enter the circulation is harmlessly discharged in the feces.

Employing rats and dogs, the analysis of central nervous tissue revealed unusually high titer of bismuth following short periods of oral dihydroxypropyl bismuthate administration. A dog receiving 10 mg. of bismuth per Kg. by mouth daily for 8 weeks revealed high tissue contents of bismuth and the absence of histological damage.

Preliminary clinical studies in the Department of Dermatology and Syphilology revealed encouraging results following oral administration of dihydroxypropyl bismuthate to syphilitic patients. Only cases of secondary and tertiary syphilis were available for experimental treatment. Patients suffering from lichen planus and warts were given dihydroxypropyl bismuthate orally with measurable benefit.

Dihydroxypropyl bismuthate by oral administration furnishes systemic absorption of bismuth in therapeutically active amounts. In four cases of secondary syphilis and 22 cases of tertiary syphilis, it produced varying degrees of clinical progress. This therapeutic activity was the result of continued daily oral administration. Beneficial results were obtained in cases of latent neurosyphilis. The results of 15 months of clinical trial are reported (10).

Quantitative analysis of urine, blood and spinal fluid established bismuth concentrations in those fluids as high as or higher than those under standard intramuscular therapy. Daily urinary elimination of bismuth following oral ingestion of dihydroxypropyl bismuthate by 50 patients varied from 2 to 20 mg. Blood bismuth levels averaged 0.185 mg. % during administration of dihydroxypropyl bismuthate which is of the same order as obtained when bismuth subsalicylate is given intramuscularly. Blood concentrations of 0.350 mg. % bismuth were not uncommon following the oral administration of the soluble compound, while the blood level produced by bismuth subsalicylate exhibits a cyclic variation of from 0.10 to 0.20 mg. % bismuth between injections. Spinal fluid concentrations of bismuth following dihydroxypropyl bismuthate range from 100 to 300% of those in the blood. These levels are noticeably higher than those following intramuscular therapy and their efficacy may be traced by the usual spinal fluid observation.

DISCUSSION

An entirely new series of bismuth compounds has been prepared. This series contains members which have suggested themselves for therapeutic application. One member has been studied in detail both pharmacologically and clinically, while preliminary animal experimentation reveals that several other members are suitable as therapeutic agents.

Desirable physical and chemical properties have been combined to form the members of

this series. Since these compounds may be prepared in pure form, chemical studies have shown their stability in all body fluids. Because the bismuth is present as the bismuthate and not as a free ion, members of this series do not coagulate and precipitate protein. Also, due to this chemical arrangement, the members are stable within a *p*H range of from mild acidity to extreme alkalinity. Such stability allows these compounds to pass through the stomach and remain soluble in the normally alkaline intestinal fluids. This property makes possible intestinal absorption in amounts previously impossible. It now appears that wider margins of safety, as well as high and more uniform blood levels of bismuth, are possible during oral administration as compared to the standard intramuscular route previously employed. Compounds of this series are suitable for intramuscular injection, with both the advantages and disadvantages of the water-soluble group.

SUMMARY

1. A new method describing the preparation, isolation and purification of a series of new bismuth compounds is reported.

2. This series furnishes members, the solutions of which are neutral, are uneffected by mild acidity or alkalinity, and are stable in body fluids.

3. One member of this series has been studied pharmacologically and clinically; two other members have been subjected to preliminary pharmacological experimentation.

4. As a protection to the public, an application for a United States patent has been made by the Committee on Patents and Copyrights of the University.

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A Distinctive Test For Sulfadiazine*

By Harry W. Raybin†

A simple test has been found that will distinguish sulfadiazine from the other common sulfonamides. The sulfonamides giving negative tests included sulfanilamide, sulfapyridine, sulfathiazole, 4-methylsulfathiazole, sulfaguanidine, sulfacetimide, sulfasuxidine, 4-methylsulfadiazine, 4,6-dimethylsulfadiazine and sulfapyrazine, the para-isomer of sulfadiazine. Negative tests were also given by 4-methyl-2-aminopyrimidine, 4,6-dimethyl-2-aminopyrimidine and 2-aminopyrazine.

While the test may be performed on sulfadiazine, the 2-aminopyrimidine moiety is responsible for the reaction. Therefore it is preferred to heat the sulfadiazine to obtain a sublimate of 2-aminopyrimidine.

The 1943 New and Non-Official Remedies gives the details for decomposing sulfadiazine to give 2-aminopyrimidine. However, for the purpose of this test it suffices to heat the sulfadiazine (0.1 Gm.) in a small test tube over a low flame until the sulfadiazine melts and a ring of sublimate appears. After cooling, some of the sublimate is scraped off and used directly in the test without further purification.

* Received Aug. 31, 1943 from the Chemical Laboratory, New York City Health Dept., Bureau of Food and Drugs.

This test has been incorporated into the Supplement of the U. S. P. XII in the Monograph on Sulfadiazine.

† Acknowledgment is made to Dr. Richard O. Roblin, Jr., for the gift of 4-methyl and 4,6-dimethylsulfadiazines and corresponding aminopyridmidines and to Dr. R. C. Ellingson for the 4-sulfapyrazine, and the 2-aminopyrazine.

The encouragement of Harry Taub, Chemist in Charge of the Chemical Laboratory of the New York City Health Department is appreciated.

EXPERIMENTAL

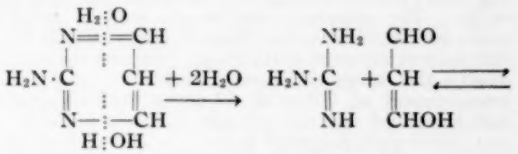
Procedure.—A 5% alcoholic solution of resorcinol serves as the reagent. Dissolve a few milligrams of the 2-aminopyrimidine sublimate in 1 ml. of the reagent and add 1 ml. of concentrated sulfuric acid. The temperature rises to about 75° C. and a deep red color is produced. If the reaction product is diluted with 25 ml. ice water and neutralized with ammonia, the color changes to blue with a reddish tinge. If sufficient aminopyrimidine (0.1 Gm.) has been used, and the reaction product poured on some cracked ice and neutralized with sodium bicarbonate, a purple solid may be isolated, which gives a deep blue with ammonia or alkali. The acid form is reddish brown with a bronze reflex similar to fuchsin.

The test is very delicate, and the red color may be obtained with 0.01 mg. of 2-aminopyrimidine. The test may be performed, if desired, on the material from the melting point determination of sulfadiazine by heating the melting point tube over a tiny flame to obtain a microsublimate. The ring of sublimate is cut off and the capillary crushed in a test tube with a glass rod, and the test performed as above.

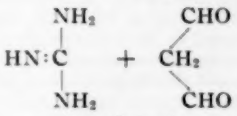
Results similar to those with resorcinol are obtained with other phenols (*e. g., phloroglucinol*) and with other acids as hydrochloric acid (heat), but the method described is recommended.

The test must naturally be used with proper regard for other known color tests involving resorcinol and acids with various aldehydes, ketones and carbohydrates. In the case of sulfadiazine, the melting point (252°–256° C.), the melting point of the 2-aminopyrimidine sublimate (126°–7° C.) and the colors in the resorcinol test are quite conclusive identification. The formaldehyde sulfoxylate of sulfanilamide (aldenil) gives red resinous precipitates of the well-known Baeyer phenolformaldehyde type (and no blue color change with acids).

It is possible that the color is due to the formation of malonic aldehyde formed by hydrolysis of the 2-aminopyrimidine and to a subsequent condensation of this aldehyde with resorcinol.



(enol forms)



(keto forms)

A study of the chemistry of the colored reaction product has been undertaken.

SUMMARY

A distinctive test for sulfadiazine or 2-aminopyrimidine consists in treating with resorcinol and sulfuric acid to produce a red color, blue with a reddish tinge with ammonia (1).

REFERENCES

(1) Circular 447, U. S. P. Revision Committee (1940–1950), p. 1625.

Book Reviews

Optical Crystallography, by ERNEST E. WAHLSTROM, University of Colorado. John Wiley and Sons, New York, 1943. 206 pp., 13.5 x 21.5 cm. Price, cloth, $3.00.

The study and identification of small amounts of chemical material by means of the petrographic microscope is a rapidly expanding field of endeavor. A few of the more progressive colleges of pharmacy already include courses in chemical microscopy in their curricula and as pharmaceutical education advances many more will come to realize the importance of this field. Not only does chemical microscopy offer a rapid method for the identification and the determination of the purity of both inorganic and organic chemicals, but it also contributes greatly to research in those branches involving the study of chemical substances occurring in minute amounts in material of vegetable and animal origin. Neither the modern pharmaceutical chemist nor the modern pharmacognosist should be without training in the use of the petrographic microscope.

Advancement in the application of the petrographic microscope to problems in chemistry, pharmacy and medicine has been somewhat slower than is really warranted. The early workers who perfected polarizing microscopes were mineralogists, and while they developed the fundamental theories to a high state, it was necessary for the modern worker in other fields to wade through a maze of mathematical data, often highly discouraging to him in his attempt to apply petrographic methods to his problem. There is unfortunately no royal road to optical crystallography. Such phenomena as refractive index, optic sign and interference figures, highly useful in the identification of crystals, cannot be properly interpreted without a thorough understanding of the effect of crystals on the light rays passing through them.

Professor Wahlstrom's book, more than any other this reviewer has seen, presents a highly detailed and correlated discussion of optical crystallography with a minimum of mathematical interpolation. In many instances he begins with a discussion of the simple properties of light (often omitted by other authors) and develops it into a discussion of their relation to the more complex optical effects observed

by the crystallographer. He accomplishes this end by means of many excellent illustrations designed to emphasize in the mind of the reader the importance of a three dimensional visualization of the relationships between optical directions and crystal directions.

Geometric crystallography and the physical properties of crystals are briefly discussed. An entire chapter is devoted to elementary optics to assure that the reader has sufficient fundamental grounding before proceeding into the more complex crystal optics. The polarizing microscope and its accessories are described in two chapters with emphasis on the theories involved in their use. Various chapters discuss the optics of isotropic substances and the measurement of index of refraction, the uniaxial indicatrix and uniaxial crystals in plane-polarized and convergent-polarized light, sign determination in uniaxial crystals, biaxial crystals and the triaxial ellipsoid and biaxial crystals in convergent-polarized light, the determination of optic sign in biaxial crystals, dispersion in biaxial crystals and the microscopic examination of nonopaque substances.

Not only should the book serve excellently for the purpose for which it is designed, namely, as a textbook for the review of the principles of optical crystallographic theory, but it should contribute much to the advancement and popularization of chemical microscopy through its simplification of these optical principles and its presentation of them in a readily understandable form.—ELMER H. WIRTH.

Physical Biochemistry, by HENRY B. BULL. John Wiley and Sons, New York, 1943. iv + 347 pp., 13.5 x 21.5 cm. Price, $3.75.

This book was written to fill a long-felt need for a textbook of fundamental physical chemistry especially written from the viewpoint of students of biology and premedical students of biochemistry. Most of the traditional material covered in physical chemistry courses is present, but the arrangement and the outlook are different.

Biology and biochemistry students frequently fail to see the relationship between theory and practice. Seeing this failure, Professor Bull has used biological illustrations wherever possible. For example, on page 20 the author has illustrated the energetics of osmosis by a diagram showing the living cell doing osmotic work, and beginning on page 83 the Hofmeister ion series is explained in terms of ionic hydrations coupled with biological considerations.

Much of the traditional physical chemistry of gases and liquids has been omitted or incorporated into various parts of the text without the loss of fundamental concepts. On the other hand, a great deal more emphasis is placed on colloidal phenomena. It is this last phase that is particularly well executed since Professor Bull is a recognized authority in colloidal chemistry especially as applied to the lipids.

Such relatively modern topics as bound water transition-state energy, and the relationship between molecular structure and dielectric properties are adequately discussed for an elementary text.

The student will find this an interesting and understandable text; and the research worker and teacher will find it stimulating, for it places biochemistry on the highest scientific plane.—M. W. GREEN.

The Examination of Waters and Water Supplies, by ERNEST VICTOR SUCKLING. The Blakiston Company, Philadelphia. Fifth edition, 1943. x + 849 pp., 63 illus., 15 x 24 cm. Price, $12.00.

This book is a complete handbook on the examination of potable waters and the pharmaceutical chemist or bacteriologist who has occasion to examine such waters will find it to be a helpful guide.

This handbook is divided into 8 sections, the individual parts being: Purposes of the Examination of Waters and the Relation of Geology to Water Supplies; the Inspection of Waterworks and the Sources of Water Supplies; the Qualities Required of Waters in Their Use for Drinking, Domestic and Industrial Uses; Interpretation of Results of Physical, Biological and Chemical Examinations; Analytical Processes and Methods; the Analytical Characters of Waters in Relation to Their Geological Origin; Bacteriological Examination of Water; and the Purification and Treatment of Water.

Although a British publication, the basic principles described in this reference book will be applicable to most American practice. In addition, American practices which are materially different from the British are singled out by the author. It is surprising, however, to find no mention of the American practice of using hexametaphosphates for water softening, preventing boiler scale and the like.

The appendix is especially valuable for the formulas of reagents and culture media which it contains. The appendix also contains a number of tables such as the solubility of oxygen in water as a function of temperature, specific gravities of various salt solutions, filtering speeds in sand, hardness, conversion tables, etc.—M. W. GREEN.

Biochemistry of the Fatty Acids, by W. R. BLOOR. Reinhold Publishing Company, New York, 1943. ix + 387 pp., 15 x 23 cm. Price, $6.00.

This authoritative book, one of the American Chemical Society monographs, was written to furnish "background for future effort." Due to the hundreds of literature quotations it serves very adequately in that capacity.

The monograph is divided into six extensive chapters headed: Descriptive and Analytical, Digestion and Absorption, Lipids of the Blood, the Lipids in Tissue, Lipid Metabolism, and the Lipids of Secretions and Excretions.

From the physiological viewpoint this reference book leaves little to be desired. The author treats this side of the picture with excellent judgment, and his first-hand knowledge of the early work in the field contributes much toward broadness of perspective. It is unfortunate, however, that the discussion of the chemical side of the picture is somewhat curtailed. For example, the excellent work on the synthesis of fats of known structure and the correlation of structure with physical properties has been largely omitted. Also the physical-chemical approach has been given too little attention. Although it is obvious that space could not permit a detailed discussion of recent studies on fat films by pressure-area analysis, yet the implication of this work in biology is so vast it would seem to justify at least a few pages.

Due to war conditions, the type is somewhat crowded on the page and the quality of the paper is poorer than usual. The omission of an author index is unfortunate.—M. W. GREEN.